EFFECTS OF DENSITY AND WATER AVAILABILITY ON THE BEHAVIOR, PHYSIOLOGY, AND WEIGHT LOSS OF SLAUGHTER HORSES DURING TRANSPORT

A Thesis

by

CHRISTA MARIE IACONO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2005

Major Subject: Animal Science

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Approved by:

Chair of Committee, Ted H. Friend
Committee Members, Thomas H. Welsh, Jr.
Gary Varner
Head of Department, Gary Acuff

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ABSTRACT

Effects of Density and Water Availability on the Behavior, Physiology, and Weight Loss of Slaughter Horses during Transport. (December 2005)

Christa Marie Iacono, B.S., University of Wisconsin-River Falls

Chair of Advisory Committee: Dr. Ted Friend

The aim of this study was to determine the effects of density and provision of water on behavior, stress, and weight loss in slaughter horses during transport. A 16.2-m long, single deck, semi-trailer was divided into three compartments to create high, medium, and low density (5000, 4000, and 3000 kg per compartment, respectively) groups of slaughter horses. A total of six shipments containing 23 to 30 horses per shipment were transported in June and July of 2004 for 18 to 20 h. Horses were a variety of different breeds, ages, sexes, and body conditions, but were typical of slaughter horses. Jugular blood samples were taken from five horses in each of the three density treatments immediately before loading and after unloading at the completion of each shipment. All horses were weighed at the same time as the blood samples were collected. While the truck was stopped, horses in each of two compartments received water from three automatic water bowls in each side of a compartment. The water was provided for 1-h after 8 h of transport and then again just prior to unloading. The third, non-watered compartment served as a control for each of the 1-h watering sessions. Densities selected to receive water were alternated between shipments. The aggressive behavior of the horses for the six shipments was recorded using 12 video cameras installed in the trailer. All occurrences of aggressive behavior were counted from 15-min segments of video during 2-h intervals for each horse that was visible in each density group. Density did not significantly affect ($P > 0.21$) aggressive behavior, cortisol, serum chemistry profile, dehydration, and weight loss. Aggression level did not differ ($P = 0.49$) between the first and second halves of the shipments. Individual horses, rather than density, appeared to be the cause of aggressive behavior. The watered and non-watered groups did not differ ($P > 0.54$) in terms of aggression, cortisol, serum chemistry profile, dehydration, and weight loss. Density and provision of water did not significantly influence aggressive behavior, stress and weight loss in shipments of 18 to 20 h long during warm weather.

DEDICATION

I would like to dedicate this thesis to my parents, Jane and Bill Iacono. Thank you for your endless love and support.

ACKNOWLEDGEMENTS

First of all I would like to thank Dr. Ted Friend for his assistance, advice, and encouragement throughout this project. I would like to thank my committee members Dr. Gary Varner and Dr. Tom Welsh for their help and guidance. I would like to thank Dr. Omer Jenkins for helping me sort out my data and statistics. I must thank Dr. Tom Goerke, my undergraduate advisor from the University of Wisconsin-River Falls. His enthusiasm and assistance in helping me find the right grad program in animal behavior went way beyond the call of duty of a typical undergraduate advisor. I also would like to thank the USDA-APHIS Veterinary Services for funding this project.

To my family, thanks for being there for me and bringing me home several times a year to see everyone. Your emotional support, advice, and love helped carry me along through this whole process. I am so grateful for my boyfriend, Josh, who dropped his life in California and moved to Texas so that we could be together while I worked on my master's degree. The sacrifices he made for me are innumerable and greatly appreciated. Thanks to my best friend Carrie for all of her support, good humor, and advice. No one could ask for a better friend.

Thanks to the grad students and researchers working under Dr. Friend. A special thanks to Greg who helped me with statistics, computer problems, and reminding me when I needed to get my tuition waiver and other paperwork done before the start of each semester. I would also like to thank Pete for introducing me to the wildlife and fisheries gang and for always having a great sense of humor. Ryan, Tracy, Nicole, and Heidi, your assistance and guidance were much appreciated. I have lots of fond memories of our adventures during the many horse hauling road trips.

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INTRODUCTION

United States Department of Agriculture (USDA) inspection reports indicated 63,000 horses were slaughtered in the United States in 2003 (USDA, 2004). At present, three federally inspected horse slaughter plants exist in the United States. Two are located in Texas and one recently opened in Illinois. Legislation was passed in 1996 in the United States (1996 Farm Bill. Sec. 901-905) to authorize the regulation of the commercial transport of horses transported to slaughter. The USDA, APHIS Veterinary Services, was directed to develop these regulations and standards. Since 1996, studies have examined certain aspects of long distance transport of loose horses. Potential issues of concern relating to the horses' well-being have arisen from these studies.

One issue of concern is that transported slaughter horses may become severely dehydrated during transport. The studies conducted by Friend et al. (1998), Friend (2000), and Stull (1999) demonstrated that horses may become extremely dehydrated and consequently lose weight during long distance transport. Results from those studies were used by the USDA to formulate regulations that allow for horses to be transported a maximum of 28 h without food, water, and rest. Due to the central location and scarcity of the slaughter plants, travel approaching the maximum allowed by law for slaughter horses is common. Long distance travel along with the sale process prior to transportation is likely stressful for horses. Warriss (1990) found that pre-slaughter cattle lose weight during long intervals of transport primarily due to dehydration and that total time traveling rather than distance was the most important factor attributing to that

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This thesis is in the style and format of the Journal of Animal Science.

weight loss. It seems likely the transport process and its effects on the horses would be similar to that of cattle. As a result, dehydration in transported slaughter horses is a cause of concern for the welfare of the horses and the associated weight loss causes a financial loss to the owner of the horses.

In addition to the issues of dehydration and weight loss in transported slaughter horses, another concern is that currently no regulations exist on the maximum density allowed for transporting slaughter horses onboard commercial semi-trailers. Current USDA regulations are performance based and require that "each equine has enough floor space to ensure that no equine is crowded in a way likely to cause injury or discomfort (Federal Register, 2001)." This statement leaves room for different interpretations of what is acceptable. Consequently, horses may be packed tightly into compartments, leading to several potentially stressful situations. In high density conditions, it is likely that horses are stressed due to reduced air flow, not being able to choose a comfortable body posture, increased dehydration, not being able to get away from aggressive individuals, and if a horse were to lie down, it could be trampled or trapped on the trailer floor.

LITERATURE REVIEW

Density

Several studies have examined the effects of different densities of horses onboard a horse trailer during transport. Collins et al. (2000) found the number of horses that fell and the number of horses injured was higher in a high density compartment as compared to a low density compartment. It was observed in that study that it was hard for the horses to rise once they had fallen in the high density group. In a survey of nine trailer loads of slaughter horses, horses in higher density compartments had significantly fewer injuries, but higher physiological indicators of stress as compared to those in smaller density compartments (Stull, 1999). Gibbs and Friend (2000) determined that having a large compartment (2.4 m X 7.2 m) with a large number of horses (n = 12) allowed more horses to drink than smaller compartments (2.4 m X 3.6 m) with fewer horses (n = 4 and 6). The larger compartment allowed time for the horses to maneuver in order to gain access to the water troughs. Horses did not show a difference in physiological measures of dehydration, such as total serum protein, sodium and chloride levels between densities of 4 horses per 8.64 m^2 and 6 horses per 8.64 m^2. No weight data were available for that study to determine the density in terms of kg per area.

The effects of density during transport have been more extensively studied on cattle and sheep. Cattle transported for 4 h in the highest of three density groups (600 vs. 200 and 300 kg/m^2) had increased cortisol, glucose, creatine kinase activity, and carcass bruising as compared to the low density treatments (Tarrant et al., 1988). Also, several cattle in the high density group in that study ended up going down and often became trapped and(or) caused other cattle to go down. Another study by Tarrant et al. (1992)

determined the effects of high density stocking on transported cattle for 24 h. Similarly to the short distance trip, when transported for 24 h, the cattle in higher density groups had elevated cortisol and glucose concentrations, and cattle that went down during transport were trapped more frequently in high density compartments as compared to those in a less dense compartment. In a study with lambs transported for 24 h, lambs in the higher density (0.909 m^2/100 kg vs. 0.613 m^2/100 kg) lay down and rested less and had an increase in creatine kinase as compared to the lower density treatments (Knowles et al., 1998). It was recommended in these studies that the animals be transported at densities lower than the high densities tested in order to improve the welfare and meat quality of the animals.

Orientation

Several studies have examined orientation (direction horse is facing during transport) preference of horses being transported, but had mixed results. Cregier (1982) believed that forward facing horses were uncomfortable traveling forwards because they must constantly protect their head and chest by relying on their hindquarters for balance and by holding their head in an unnatural position to avoid impact. The rear facing position was believed superior because the fleshy rump of the horse could more easily brace against impact, the horse could balance over its forequarters, and it would not have to hold its head in a strained position to prevent injury (Cregier, 1982). Several researchers have since attempted to determine a scientific basis for the importance of rear facing orientation during transport. Smith et al. (1994a) and Kusunose and Tonkai (1996) determined that when given the option, horses spend significantly more time facing backwards in a trailer as compared to facing forward. The trailer used by Smith et al. (1994a) was a four-horse stock trailer with one horse transported per trial. The trailer used by Kusinose et al. (1996) was a six-horse trailer modified to contain two horses in a 3.5 X 2.1-m compartment. Research has also shown that average heart rate is lower in transported horses facing backwards in a two horse straight load lorry (Waran et al., 1996). However, a study conducted by Clark et al. (1993) did not find a significant difference in the heart rates of horses facing forwards vs. backwards, but did find that rear facing horses were able to maintain balance better than those facing forwards in a two-horse side by side straight load trailer. Smith et al. (1994b) determined that heart rates were not different between horses facing forwards or backwards when transported singly in a four-horse stock trailer. Movement indicative of balance of the horses in different orientation positions was not significantly different when transported in a 16-m single deck commercial trailer divided into four 3.7 X 2.4-m stalls, each containing one horse (Toscano and Friend, 2001). Collins et al. (2000) determined that 18 to 21 horses transported loosely on a 16-m commercial trailer did not show a preference for facing toward (47.5%) or away (40.7%) from the direction of travel. Horses had strong individual preferences to trailer orientation (Smith et al., 1994a; Toscano and Friend, 2001). The use of different types and sizes of trailers, whether or not horses can look out the back of the trailer, number of horses per compartment, previous individual horse traveling experience, and individual preferences by horses could account for the variability of results.

Elevated Head Postures

Studies have examined the effect of a long term elevated head posture on the health of horses. Slaughter horses may be forced to keep an elevated head posture in high densities during transport due to limited maneuvering room. Stull and Rodiek (2002) determined that horses that were cross-tied for head restraint for long distance transport had significantly elevated white blood cell counts, neutrophil to lymphocyte ratios, glucose, and cortisol levels as compared to horses transported loosely. A non-transport study determined horses that were confined with their heads elevated for 24 h developed an increased number of bacteria in the lower respiratory tract and showed a decrease in tracheal mucociliary clearance whereas horses that could lower their heads showed an accelerated rate of tracheal mucociliary clearance (Raidal et al., 1996). Of twenty four confined non-transported horses not able to lower their heads for 24 or 48 h, 21 mares showed increases in the number of bacteria in transtracheal aspirates, eight mares showed signs of mild respiratory disease, and three mares showed signs of systemic illness (Racklyeft and Love, 1990). These changes were reversed after the horses were able to lower their heads. Elevated head postures such as those in horses that are tied or in high density groups during transportation could potentially lead to a higher incidence of respiratory disease and distress.

Stress

Studies have demonstrated that transport is stressful to horses as determined by physiological measures. Cortisol has been a common measure of transport stress in horses. Four horses transported in shifts with a two-horse bumper pull (Smith et al.,

1996) and 30 horses transported in a commercial trailer (Friend, 2000) showed increases in cortisol concentrations post-transport. Clark et al. (1993) determined that horses in a two-horse trailer had an increase in cortisol concentrations and heart rate. Heart rate also increased in pregnant mares transported for four hours during transport (Mars et al.; 1992). Along with increases in cortisol concentrations post-transport, horses have shown signs of stress such as increased levels of sodium and glucose concentrations (White et al., 1991), increased neutrophil to lymphocyte ratios, and white blood cell counts (Stull, 1999; Stull and Rodiek, 2000; Stull et al., 2004). Baucus et al. (1990b) compared the physiology of 15 mares transported for 12 h at the pre-ovulatory stage of estrus with 15 non-transported controls. The transported mares had higher concentrations of cortisol and plasma ascorbic acid as compared to the controls, but their reproductive functions were not affected by transportation. In a similar study, pregnant horses were transported to determine the effects of transport on early embryonic death, and plasma ascorbic acid (Baucus et al., 1990a). Horses in that study had elevated levels of cortisol and ascorbic acid, but transport had no effect on early embryonic death. Most of these stress transport studies involved healthy horses. It is likely that many slaughter horses would be in a compromised state of health and would therefore show higher responses than what was determined in the stress response studies of healthy horses.

Dehydration and Weight Loss

Horses need between 18.93 and 75.71 L of water per day depending on age, size, environmental temperature, relative humidity, type of feed provided, and type of work required of the horse (Hinton, 1978). Delahanty (1966, as cited in Hinton et al., 1978) concluded that a decrease in body weight can be a good indicator of dehydration and that a 4, 6, and 8 percent loss in body weight was indicative of mild, moderate, and severe dehydration. Death occurred when 15 to 17% of the body weight was lost. Additional studies have examined the association of dehydration and weight loss in various environmental conditions. A study was conducted where horses were housed in metabolism stalls and deprived of food and water for 8 d in cool weather (Tasker, 1967). After 8 d of water and food deprivation, horses in that study lost an average of 10% of their body weight. Sneddon et al. (1991) determined that desert dwelling horses in high ambient temperatures can easily tolerate water deprivation to the point of a 12% weight loss which occurred after 72 h. Similarly, when horses were deprived of food and water for 72 h in high environmental temperatures, they lost an average of 10.7% of their body weight (Carlson et al., 1979). After the period of dehydration, Carlson et al. (1979) provided water to the horses for 1 h and the horses regained 62% of their lost body weight. Houpt et al. (2000) found that pregnant mares with restricted water intake (less than half the amount the horses would drink freely) for 3 weeks become dehydrated and lost a mean of 8.7 ± 1.5%. These studies indicate that healthy horses can tolerate water deprivation, recover relatively quickly, and in cooler weather, they can go remarkably long periods without water. It is important to note however, that the horses in these studies were healthy and in good condition before the water deprivation. Slaughter horses would likely be in a poorer state of health and condition and would therefore not be able to go as long without water.

Studies have determined methods utilized by horses to cope with hot temperatures and how their physiology is altered when handling temperature changes in the environment. Honstein and Monty (1977) determined that rectal and skin temperatures were significantly higher in hot weather as compared to cooler weather and that sweating was the primary method for the horses to thermoregulate. Sweating can cause large fluid and electrolyte losses that can lead to dehydration in horses (McKeever, 1998). This loss of fluids and electrolytes likely occurs when horses sweat onboard the trailer during transport in hot weather. In support of this, studies have determined that horses become dehydrated from transport in hot weather and in trips of long duration (Friend et al, 1998; Friend, 2000; Stull and Rodiek, 2000). Studies on long distance transport stress on cattle have determined similar results as the horse transport studies. With two different groups of calves transported on trips of different durations (1 d vs. 2 d), the calves in the long distance transport group showed increased signs of dehydration, hypoglycemia, and level of respiratory disease as compared to those in the short transport group (Mormede et al., 1982). Researchers have determined that one way to help prevent dehydration is by providing water to horses during transport (Gibbs and Friend, 2000; Friend, 2000; Friend et al., 1998).

Providing Water during Transport

A study conducted by Irwin and Gentleman (1978) demonstrated that cattle transported long distance by railcar readily consumed feed and water onboard the railcar during rest periods and appeared in good condition after a 42 h trip. Cattle transported by railcar with feed and water access were compared to cattle transported by truck (Friend et al., 1981). The cattle transported by railcar in that study lost less weight (6.6% vs. 10.6%) and had fewer incidence of shipping fever (5% vs. 8%). Likewise, Gibbs and

Friend (2000) found that horses readily consumed water from wall-mounted water troughs when onboard a trailer, but the troughs used were not practical for commercial use. Our lab has since developed an onboard removable watering system that is more practical for commercial transport and only used when the trailer is not in motion. The onboard watering system utilized smaller plastic water bowls without sharp edges. The water was stored on the semi-trailer in order to allow for convenient access to the water and also to use water from a source to which the horses were accustomed. Excess water drained to the outside of the trailer and removal of residual water was simplified by the accessibility of the individual water bowls.

During each watering session, plywood plugs (15.24 cm X 35.56 cm) were removed from the side of the trailer and six water bowls were inserted (three on each side for a total of six per compartment) and secured with a spring latch. The water bowls were Lister SB NT 100 plastic float valve water bowls (Syrvet, Inc., Waukee, Iowa) mounted on a custom designed bracket. Two 550-L tanks were used to store water underneath the semi-trailer. Water was pumped through PVC pipes located on the outside of the trailer and reached the water bowls by flowing through 30-cm long tubes attached to the PVC pipe system and water bowls with quick-connect fittings. Height of water bowls from the trailer floor was 75 cm, which was determined by horse preference during a preliminary study.

Three shipments of slaughter horses were used in a preliminary study by our lab to test the onboard watering system. The trailer was divided into three compartments to separate the treatment groups. Horses in two of the compartments were given water and one compartment of horses served as the non-watered control group. Latency until first drink, number of horses drinking, and weight lost by the watered and non-watered groups during two one-hour watering sessions was determined. The results from that preliminary study showed that slaughter horses would drink onboard a semi-trailer if given the chance and were more likely to drink sooner in hot weather than cooler weather (Iacono et al., 2005). In that study, horses that had limited access to water prior to loading and consumed water in hot weather lost less weight than those that did not have water access.

Fatigue

Another possible concern is the fatigue of the horses from transport of long duration. Friend (2000) found that healthy horses transported in hot weather showed signs of fatigue after 24 h. By 28 h, even horses with access to water on the trailer were fatigued and by 33 h, transport ceased out of concern for the health of the fatigued horses. In response to the problem of fatigue from transport, a recent study was conducted that examined onboard intermittent rest and trailer cleaning on the inflammatory and stress response of long distance transport in healthy horses (Oikawa et al., 2005). Horses that were given longer resting periods of 2 h every 5 h of transport and had their trailers cleaned at rest stops showed lower levels of transportation and respiratory stress. Providing onboard rest periods to slaughter horses during transport may help reduce fatigue, but more studies are needed in this area.

Objectives

One major objective of this study was to determine the effect density of slaughter horses had on aggression, dehydration, and stress. The current study aimed to test the hypothesis that slaughter horses would have fewer or less severe aggressive encounters and consequently, be less stressed during transport when loaded at medium or low density as compared to high density. Horses in lower density compartments would also likely become less dehydrated and stressed than those in a higher density group. Another hypothesis was that the first half of each shipment was likely to have more aggressive encounters between horses as compared to the second half of the shipment as a result of fatigue.

Another major objective was to determine the extent to which the watering system used in the preliminary study would also be utilized by the horses in the current study. The horses used in this study were hypothesized to lose less weight if water access was available, and would show fewer signs of dehydration and stress.

MATERIALS AND METHODS

Equipment

The trailer used to transport the horses was a custom built 16.2-m long X 2.4-m wide X 2.62-m high, single-deck, slat sided trailer (Barrett Trailers, Purcell, Oklahoma). The front two compartments were 5.2 m X 2.4 m and the rear compartment was 5.5 m X 2.4 m. The inside of the trailer was lined with plywood to a height of 1.46 m to prevent horses from becoming injured and also to prevent damage to the semi-trailer. The floor was made with a "5-bar tread plates" pattern and the deck plate was crimped to create four 2-cm high triangular ridges, spaced 42-cm apart that ran the length of the trailer for traction. The trailer was ventilated by eight pop up air vents (22.9 cm X 8.9 cm) spaced equally along the roof and also by two 15.24-cm wide air gaps in slats that ran the length of the sides of the trailer starting at 1.83-m above the floor.

Four Capture 1/3" CCD outdoor bullet cameras (Richardson Electronics, Houston, TX) were mounted in the top four corners of each of the three compartments. The cameras fed to four or ten channel multiplexer recording devices (GE-Interlogix Kalatel Division, Corvallis, Oregon) stored underneath the trailer. The multiplexers were placed in storage containers that provided an environment of forced air ventilation with filtered air. Air suspension made from two bicycle inner tubes was used to isolate the multiplexers from transport related vibrations. Video was collected for the full duration of the trip. The temperature and humidity inside the trailer was recorded every 5-min with four HOBO H-8 (Onset Computer, Bourne, MA) temperature data loggers. Two temperature data loggers were mounted in the front and rear compartments near the roof and the other two were mounted 40-cm above the floor on the sides of the middle and rear compartments.

Experimental Design

The three compartments on the semi-trailer were used to create groups of high, medium and low density. The density was based on weight per compartment. The target pre-transport weights were 5000, 4000, and 3000 kg for the high, medium and low density compartments, respectively. Canada has a recommended maximum density for horses of different weights in The Canadian Code of Practice (CARC, 2001). The Canadian Code of Practice recommends a maximum density for 450-kg horses to not exceed 400 kg/m^2 (CARC, 2001). In line with the Canadian Recommended Codes of Practice, the average weight per horse in the high density group in this study was 445 kg and the maximum average density was 397 kg/m^2. The high, medium and low density groups were rotated into different compartments for each trip to help control for compartmental biases.

Six shipments of slaughter horses were transported during the months of June and July of 2004. The first shipment of slaughter horses originated from La Grande, Oregon and was dropped off in Hudson, Colorado (n = 23). All of the Oregon horses remained in Colorado for four days. Horses originating from Colorado, along with about one-third of the previously transported Oregon horses, were then transported to a Texas slaughter plant (n = 26). Transport of the Oregon horses from Colorado to Texas was considered a separate shipment (shipment three). The remaining four shipments originated from Hudson, Colorado and ended at one of the two slaughter plants in Texas (n = 26, 30, 25, 27). The duration of each shipment ranged from 18 to 20 h. The length of time animals were housed together and supplied with food and water before transport varied. Typical of shipments of slaughter horses, horses were of a variety of different breeds, ages, sexes and body conditions. Prior to loading, numbers were placed on the backs of the horses with grease livestock markers to facilitate identification during analysis of the video data.

Watering Sessions

Horses in two of the compartments received water, with the third non-watered compartment serving as a control. The two densities selected to receive water were alternated between shipments to help control for density biases. Two one-hour watering sessions were given to the horses, with the first one starting after eight hours of transport and the last just prior to unloading on arrival at the slaughter plants or Hudson, Colorado (shipment 1). The horses were weighed before and after transport to measure weight loss. Certified scales used for weighing at auctions and slaughter plants were utilized to weigh the horses. A portable TI-500 Series Digital Indicator scale (Transcell Technology, Inc., Buffalo Grove, IL) provided by the researchers was used to weigh the horses in Hudson, Colorado, when certified scales were not available. Only the weights of horses from shipments one, four, five and six were used for the weight data due to inaccurate weights from shipments two and three.

Behavior

The occurrence of aggressive behavior was determined for the different density treatments. When the aggression data were initially analyzed, a 30-min segment of video of each horse was analyzed every two hours for two high, medium, and low density treatment groups that were spread across the six shipments. Due to the difficulty of finding 30 min of continuous video in which a focal horse was visible, sampling was changed to 15-min segments for every 2-h interval. The 30-min segments were still utilized in the data by dividing the bouts of aggression by two in order to get a number representative of 15 min. The first 15- or 30-min of each 2-h interval was used for collecting aggression data. If the truck was stopped during the first 15- or 30-min of a 2-h interval, then the data was collected during the next available time during that 2-h interval that the truck was moving. Also, if a horse was not visible during the targeted 15- or 30-min segment, then the video was searched for a different 15- or 30-min segment within the selected 2-h interval in which the horse was visible. If a horse was not visible at all during the 2-h interval, then the horse was not used for the aggression data for that specific 2-h interval. For every visible horse, the incidence and severity of all agonistic behavior for each 15- or 30-min segment was coded using one of the following four categories: threat, bite, attempt to bite, and unable to classify.

Threat: Aggressor had pinned ears and made a slightly threatening gesture such as stretched out the neck toward the recipient, but the recipient did not respond to the threat.

Bite: Aggressor had pinned ears and teeth were seen in contact with the recipient's body.

Attempt to bite: Aggressor had pinned ears and reached out to bite the recipient, but the aggressor did not make contact with the recipient's body.

Unable to classify: Aggressor made a gesture towards the recipient with ears pinned, but researcher could not tell if the aggressor was threatening or biting due to a bad camera angle or other viewing difficulties.

The multiplexer recorders were linked to PC computers and the recorded video was viewed using WaveReader 2.9 (GE Security, Corvallis, OR). The investigator (C. Iacono) recorded the aggression behavior for all of the shipments. Once the data were collected, all categories of agonistic behavior were combined to calculate the total number of aggressive encounters per compartment. That mean was divided by the total number of visible horses per compartment to get mean bouts of aggression per visible horse per 15 min.

The accuracy and precision of the investigator who analyzed the video was determined. The investigator watched nine 15-min segments spread across the six shipments and recorded the agonistic behavior for three horses that were representative samples. Those nine test segments were from different shipments with three tests per density. After the test segments were completed and all the data were recorded from the shipments, the investigator watched the same test segments again without knowing the previous results. The Pearson correlation coefficient between the first and second analysis of the test segments was 0.968.

Physiological Measures of Stress and Dehydration

Jugular blood samples were taken by venipuncture from five randomly selected horses in each of the three density treatments, for a total of 15 horses per shipment. No history was known on the horses chosen for blood collection. Blood was drawn from the selected horses before transport and from the same horses immediately after unloading at completion of each shipment. The horses were either restrained with a halter and lead rope or in a chute for blood collection. If a horse that was chosen for blood collection acted in a manner that could cause injury to itself or the researcher while drawing blood, a different horse was selected. Vacutainers, containing PST gel and lithium heparin and 20-guage needles, were used to collect blood. The blood was placed on ice until centrifugation. The blood was centrifuged within 8 h after the blood was collected and then the serum and plasma was separated. The serum was saved and put on ice where it remained for 4 h to 5 d. When cow serum was stored at -20˚C and 4˚C for 8 d, cortisol concentrations remained the same after 1 h, 2, 4, and 6 d of storage (Reimers et al., 1983).

An SMA-12 panel was run by the Texas Veterinary Medical Diagnostic Laboratory on the pre- and post-transport blood samples to determine serum protein, albumin, calcium, phosphorus, glucose, blood urea nitrogen (BUN), total bilirubin, creatinine, alkaline phosphatase (ALP), creatine kinase (CK), aspartate aminotransferase AST (SGOT), globulins, gamma glutamyl transferase (GGT), and albumin/globulin (A/G) ratios. An electrolyte profile also conducted by the Texas Veterinary Medical Diagnostic Laboratory determined the sodium, potassium, Na/K ratios, and chloride concentrations for each horse before and after transport. Cortisol and aldosterone concentrations pre- and post-transport were determined by our lab via commercially available antibody-coated-tube RIA kits (Diagnostic Products Corporation, Los Angeles, CA). Duplicates of each plasma sample from this study were assayed and results were determined by computer from a logit-log representation of the calibration curve. The net counts were determined by taking the mean counts per minute and then subtracting the mean non-specific binding counts per minute for the two samples. After the net counts were calculated, the percent bound was calculated by taking the net counts and dividing them by the net maximum binding counts. The percent bound was compared to the calibration curves for each standard curve for cortisol and aldosterone. A maximum difference of 12% between duplicate samples was used to determine if samples needed to be assayed again.

Statistical Analysis

Each compartment served as the experimental unit for the weight, aggression, and physiological data. Treatment effects in weight loss, aggression, blood chemistry measurements, cortisol, and aldosterone were determined by GLM analysis of variance (SAS 9.0, SAS Institute Inc., Cary, NC). The trip, water, density, and water interaction with density were factors in the analysis. When a treatment effect ($P < 0.05$) was detected by the model, mean separation was done using an LSD procedure. The correlation for the viewer was determined by a bivariate Pearson correlation procedure (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL).

RESULTS

Temperature and Behavior

Shipment One - Oregon to Colorado. The average temperature for the inside of the trailer was 21˚C, but ranged from 8 to 31˚C, with a mean relative humidity of 48%. There tended to be less aggression in the medium density as compared to the high and low density treatments (Figure 1; Table 1).

Shipment Two - Colorado to Texas. The average temperature for the interior of the trailer was 22˚C, with a range of 13 to 34˚C and a mean relative humidity of 73%. No behavioral data were available for that shipment due to problems with cameras and malfunction of the lighting system within the trailer (Table 1).

Shipment Three - Colorado to Texas. The mean temperature inside the trailer was 24˚C, with a range of 17 to 34˚C and the average relative humidity was 52%. No behavioral data were available for the high density treatment (Table 1). The medium density tended to have a higher average number of bouts of aggression as compared to the low density treatment (Figure 2; Table 1).

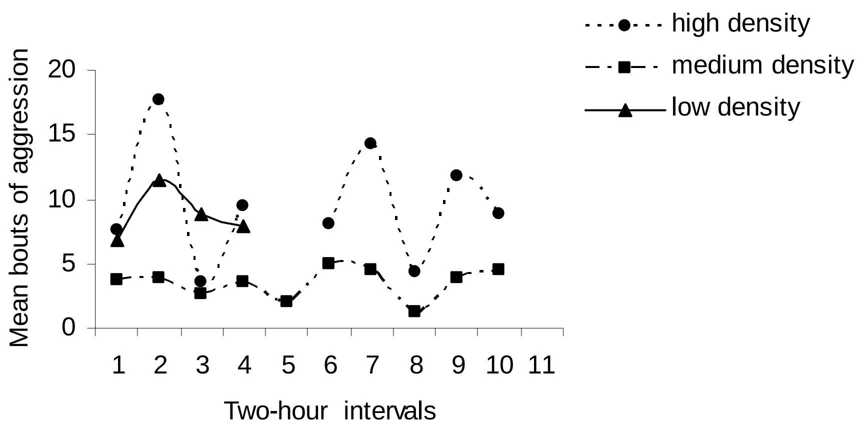

Figure 1. Mean bouts of aggression per visible horse from each 15-min segment obtained over 2-h intervals for shipment one. The number of visible horses ranged from 7 to 10 for the high density, was 8 for the medium density, and was 5 for the low density treatments. No data were available where there are missing points.

Table 1. Total number of bouts of aggression and number of horses on which the data are based () for density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments during each 2-h interval for each shipment

			Interval (h)									
Shipment	Density	Water	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20
1	H	Yes	69 (9)	142 (8)	29 (8)	76 (8)	No Data	80 (10)	100 (7)	44 (10)	94 (8)	71 (8)
1	M	Yes	30 (8)	30.5 (8)	20.5 (8)	29 (8)	16.5 (8)	40 (8)	35.5 (8)	10.5 (8)	30.5 (8)	36.5 (8)
1	L	No	34 (5)	57.5 (5)	44 (5)	39.5 (5)	No Data	No Data	No Data	No Data	No Data	No Data
2	H	Yes	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
2	M	No	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
2	L	Yes	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
3	H	No	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
3	M	Yes	61 (8)	90 (8)	32 (6)	No Data	No Data	No Data	45 (7)	33 (8)	80 (10)	No Data
3	L	Yes	3 (5)	9.5 (5)	No Data	No Data	No Data	No Data	11 (5)	7 (5)	12 (5)	No Data
4	H	No	35 (11)	30.5 (13)	25 (13)	30.5 (10)	21.5 (13)	24.5 (11)	22 (10)	14.5 (12)	28 (13)	13 (13)
4	M	Yes	5 (10)	19 (8)	16 (8)	9 (8)	32 (10)	27 (9)	15 (9)	33 (8)	12 (10)	38 (10)
4	L	Yes	12 (7)	No Data	16.5 (7)	7 (7)	13.5 (6)	8.5 (6)	11.5 (7)	2 (7)	11.5 (7)	15.5 (3)
5	H	Yes	27.5 (9)	17 (7)	19.5 (8)	32.5 (8)	6 (7)	26.5 (7)	43 (6)	34 (6)	29 (6)	25.5 (7)
5	M	No	69 (9)	15 (5)	17 (4)	44 (3)	28 (6)	23 (5)	0 (3)	3 (6)	6 (4)	12 (4)
5	L	Yes	27 (4)	8 (4)	3 (4)	19 (6)	19 (5)	25 (6)	22 (6)	19 (6)	24 (5)	16 (6)
6	H	Yes	23 (10)	34 (10)	11 (7)	14 (9)	30 (8)	31 (11)	10 (11)	3 (11)	24 (11)	No Data
6	M	Yes	13.5 (7)	15 (7)	12 (8)	16.5 (7)	20.5 (8)	20 (8)	46 (10)	10 (8)	5 (8)	No Data
6	L	No	23 (6)	13 (6)	6 (6)	19 (6)	11 (6)	1 (6)	16 (6)	11 (6)	10 (6)	No Data

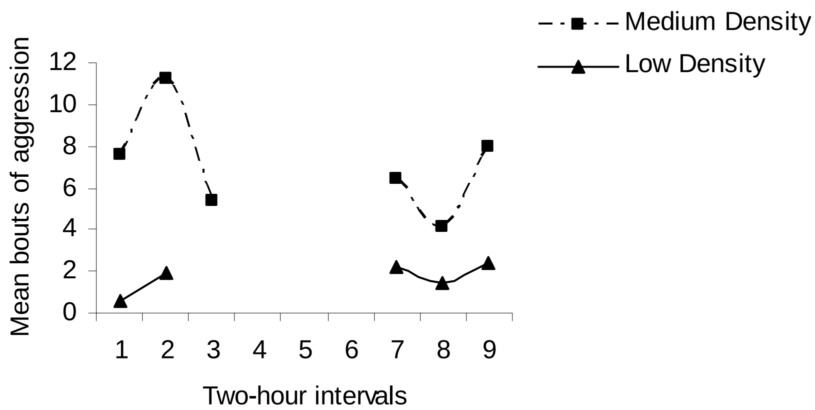

Figure 2. Mean bouts of aggression per visible horse from each 15-min segment obtained over 2-h intervals for shipment three. The number of visible horses ranged from 6 to 10 for the medium density and was 5 for the low density treatments. No data were available where there are missing points.

Shipment Four - Colorado to Texas. The inside of the trailer had a mean temperature of 29˚C and a range of 23 to 38˚C, with an average relative humidity of 52%. Average bouts of aggression did not appear to differ between the three density treatments (Figure 3; Table 1).

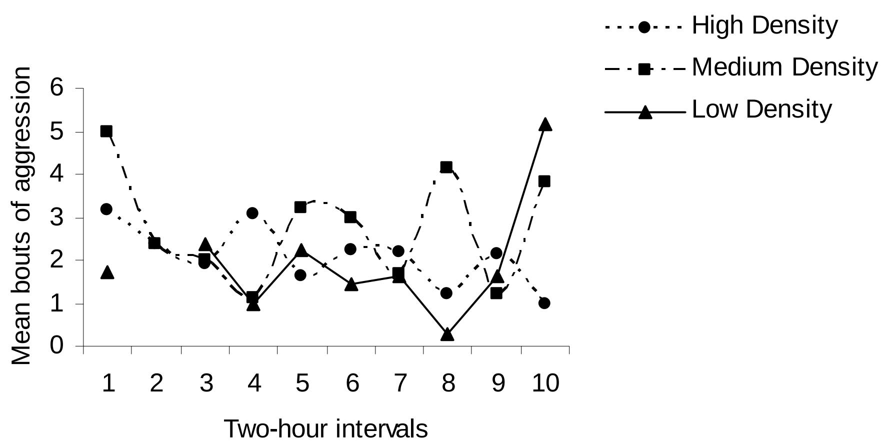

Figure 3. Mean bouts of aggression per visible horse from each 15-min segment obtained over 2-h intervals for shipment four. The number of visible horses ranged from 10 to 13 for the high density, 8 to 10 for the medium density, and 3 to 7 for the low density treatments. No data were available where there are missing points.

Shipment Five - Colorado to Texas. Temperatures inside the trailer ranged from 24 to 39˚C, with a mean of 30˚C and a mean relative humidity of 48%. No obvious differences were viewed between the three density treatments in terms of bouts of aggression (Figure 4; Table 1).

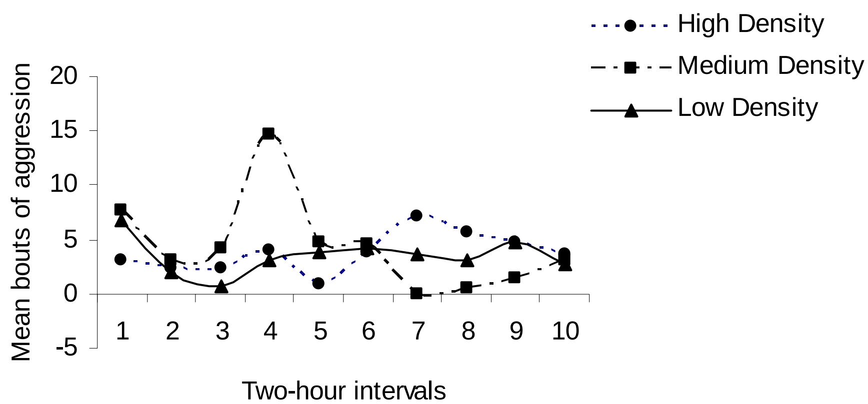

Figure 4. Mean bouts of aggression per visible horse from each 15-min segment obtained over 2-h intervals for shipment five. The number of visible horses ranged from 6 to 9 for the high density, 3 to 9 for the medium density, and 4 to 6 for the low density treatments.

Shipment Six - Colorado to Texas. The mean temperature inside the trailer was 31˚C, but ranged from 24 to 50˚C during the trip, with an average relative humidity of 45%. No obvious differences were determined between the bouts of aggression for the three density treatments (Figure 5; Table 1).

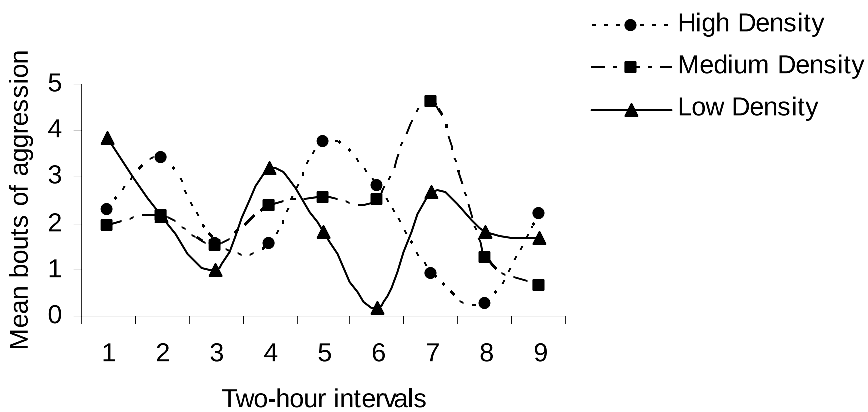

Figure 5. Mean bouts of aggression per visible horse from each 15-min segment obtained over 2-h intervals for shipment six. The number of visible horses ranged from 7 to 11 for the high density, 7 to 10 for the medium density and was 6 for the low density treatments.

Shipments One through Six. The mean temperature of the shipments was used to determine if there was a difference between horses transported in cooler vs. hotter weather. The horses in the first three shipments were transported in a mean temperature that was cooler than the horses in the last three shipments (22˚C vs. 30˚C). Weight loss, serum chemistry profile and electrolyte concentrations did not differ between the horses transported in the cool and hot weather. No significant differences were observed between the treatments when only the hot weather shipments were analyzed for density ($P = 0.36$) and water ($P = 0.13$) effects.

A few problems were encountered for the data collection on aggression. During shipments two and three, the lights inside the trailer did not work so behavioral data were not available when it was dark. One to four cameras in each compartment for every shipment either lost video or became foggy so they were not usable. On average, two to three cameras per compartment were usable for at least part of the shipments with the exception of shipment two and the high density compartment in shipment three, during which the video system malfunctioned. Therefore, not all horses on each trip were visible during all of the 2-h intervals.

When the effect of density on aggression was analyzed for shipments one, three, four, five, and six, no significant difference ($P = 0.82$; Table 2) was found between the three density treatments. Shipments one (high and medium densities only) and all density treatments of shipments four, five, and six were missing zero to one 2-h intervals for the duration of the shipment. Since those shipments and densities had the most complete data, those shipments were also analyzed together. No significant difference ($P = 0.79$; Table 2) was found between density and aggression when shipment one (high and medium densities) and all density groups of shipments four, five, and six were analyzed together. Aggression was also examined to determine if more aggression occurred during the first half of the shipment versus the second half of the shipment (Table 2). There was not a difference in the amount of aggression occurring during the first vs. second half of the shipments when all the shipments were analyzed **(**$P = 0.49$**)** and also when the high and medium densities of shipment one along with all densities of the last three shipments were analyzed together **(**$P = 0.77$; Table 2). Watered and non-watered treatments did not have different amounts of aggression when all shipments were analyzed ($P = 0.69$; Table 2) and when shipment one (high and medium densities) and all density groups of shipments four, five, and six were analyzed together ($P = 0.94$; Table 2). There were no interactions between density and water for aggression.

Number of Horses per Compartment

Shipments One through Six. The number of horses in each compartment ranged from five to thirteen (Table 3).

Table 2. Mean (± SE) bouts of aggression per 15 min for the high (H), medium (M), and low (L) density and water access (water access = Yes, no water access = No) treatments for shipments one through six

Shipment Number	Density	Water	Overall Mean	First Half of Shipment	Second Half of Shipment
1	H	Yes	9.54 ± 1.50	9.64 ± 2.97	9.46 ± 1.68
	M	Yes	3.49 ± 0.37	3.16 ± 0.36	3.82 ± 0.66
	L	No	8.75 ± 1.00	8.75 ± 1.00	No Data
2	H	Yes	No Data	No Data	No Data
	M	No	No Data	No Data	No Data
	L	Yes	No Data	No Data	No Data
3	H	No	No Data	No Data	No Data
	M	Yes	7.13 ± 1.01	8.07 ± 1.72	6.19 ± 1.12
	L	Yes	1.70 ± 0.32	1.25 ± 0.65	2.00 ± 0.31
4	H	No	2.09 ± 0.22	2.43 ± 0.30	1.76 ± 0.27
	M	Yes	2.75 ± 0.41	2.74 ± 0.66	2.76 ± 0.58
	L	Yes	1.94 ± 0.45	1.83 ± 0.31	2.03 ± 0.82
5	H	Yes	3.80 ± 0.57	2.57 ± 0.52	5.02 ± 0.65
	M	No	4.39 ± 1.35	6.85 ± 2.10	1.92 ± 0.84
	L	Yes	3.50 ± 0.51	3.29 ± 1.01	3.70 ± 0.37
6	H	Yes	2.08 ± 0.38	2.21 ± 0.43	1.55 ± 0.58
	M	Yes	2.16 ± 0.37	1.98 ± 0.18	2.25 ± 0.88
	L	No	2.04 ± 0.37	2.54 ± 0.62	1.59 ± 0.52
1, 3,	H		4.30 ± 0.62	4.02 ± 0.95	4.60 ± 0.88
4, 5 and 6	M		3.75 ± 0.41	4.36 ± 0.71	3.18 ± 0.44
	L		3.09 ± 0.41	3.76 ± 0.71	2.41 ± 0.34
1, 4, 5, and 6*	H		2.68 ± 0.28	2.42 ± 0.23	2.86 ± 0.53
	M		3.13 ± 0.51	3.99 ± 0.94	2.31 ± 0.42
	L		2.53 ± 0.29	2.61 ± 0.44	2.50 ± 0.41

* The low density data of shipment one were not included in these calculations because more than one 2-h interval was missing

Table 3. Total number of horses and total initial weight in each density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatment for shipments one through six

Shipment Number	Density	Water	Number of Horses	Initial Weight (kg)
1	H	Yes	10	4738
	M	Yes	8	4184
	L	No	5	2474
2	H	Yes	11	No Data
	M	No	10	No Data
	L	Yes	5	No Data
3	H	No	11	No Data
	M	Yes	10	No Data
	L	Yes	5	No Data
4	H	No	13	4869
	M	Yes	10	4266
	L	Yes	7	2726
5	H	Yes	10	5145
	M	No	9	4464
	L	Yes	6	2993
6	H	Yes	11	5088
	M	Yes	10	4480
	L	No	6	2836

Physiology

Shipments One through Six. The blood physiology data were used from all six of the shipments. Some of the actual blood values were not available due to missing samples. In the case of the cortisol and aldosterone assays, a few of the blood samples were used up before an accurate concentration could be determined (Appendix A).

The density and water treatments were not different in terms of showing physiological signs of dehydration (Table 4). Change in mean cortisol concentrations were not significantly different between the density ($P = 0.21$) or water ($P = 0.75$) treatments (Table 5). The change in mean aldosterone concentrations showed no meaningful differences between the density ($P = 0.72$) and water ($P = 0.83$) treatments (Table 5). The low density horses had a lower ($P = 0.02$) AST concentration than the high and medium treatments. No other constituents in the serum chemistry profile were significantly influenced by density or water treatments (Table 6, 7, and 8). However, there was a significant interaction ($P = 0.009$) with water and density when chloride was analyzed. Meaningful patterns were not found when the interaction was examined further, so it was likely not a significant factor in this study.

Weight Loss

Shipments One through Six. Weight loss was used as an indicator of dehydration and possible density effects. Some of the individual horse weights taken in shipments two and three were not accurate so the weight data from those shipments was not used in the analysis of weight lost. Total initial weights were calculated for density and water treatments for each shipment (Table 3). The mean total initial weight per compartment for shipments one, four, five, and six was 4960 kg, 4349 kg, and 2757 kg for the high, medium and low density treatments, respectively. Density did not have an effect ($P = 0.91$; Table 5) on the weight loss of the horses. Horses with water access did not lose less weight ($P = 0.54$; Table 5) than the non-watered control horses.

Table 4. Change (final minus initial concentrations) in mean total serum protein, electrolytes, aldosterone, and cortisol concentrations (± SE) for the water (water access = Yes, no water access = No) and density (high = H, medium = M, low = L) treatments for all six shipments

Water/Density Treatments [a]	Total serum protein (g/dL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)	Cortisol (ng/mL)
Yes	0.36 ± 0.1	0.53 ± 0.9	-0.29 ± 9.0	2.17 ± 0.4	15.62 ± 8.4	13.1 ± 9.0
No	0.49 ± 0.1	0.70 ± 0.9	-0.23 ± 13.2	2.57 ± 0.7	12.49 ± 8.2	16.1 ± 9.0
H	0.48 ± 0.1	1.33 ± 1.0	-0.28 ± 16.7	2.03 ± 0.6	6.94 ± 8.8	3.5 ± 1.4
M	0.43 ± 0.0	0.85 ± 0.9	-0.30 ± 0.6	2.81 ± 0.4	20.22 ± 14.5	25.1 ± 9.0
L	0.30 ± 0.1	-0.42 ± 1.5	-0.23 ± 13.3	2.06 ± 0.7	16.57 ± 8.6	13.8 ± 9.0

[a]No significant treatment effects (P > 0.05)

Table 5. Mean weight loss per horse by water (water access = Yes, no water access = No) and density (high = H, medium = M, low = L) treatments for all horses on shipments one, four, five and six (n=105)

Water/Density Treatments[a]	Mean weight loss per horse (kg)
Yes	20.07 ± 6.6
No	21.66 ± 8.2
H	20.01 ± 8.9
M	21.11 ± 9.2
L	20.68 ± 9.9

[a]No significant treatment effects (P > 0.05)

Table 6. Change (final minus initial concentrations) in mean albumin, calcium (Ca), phosphorus (P), glucose, and blood urea nitrogen (BUN) concentrations (± SE) for the water (water access = Yes, no water access = No) and density (high = H, medium = M, low = L) treatments for all six shipments

Water/Density Treatments[a]	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)	Glucose (mg/dL)	BUN (mg/dL)
Yes	0.22 ± 0.1	-0.56 ± 0.1	-0.56 ± 0.2	28.30 ± 3.5	-0.70 ± 0.7
No	0.22 ± 0.0	-0.80 ± 0.2	-0.80 ± 0.4	34.50 ± 8.5	-0.18 ± 0.9
H	0.24 ± 0.0	-0.38 ± 0.2	-0.38 ± 0.3	36.63 ± 8.2	-0.38 ± 0.9
M	0.20 ± 0.0	-0.70 ± 0.2	-0.70 ± 0.3	24.1 ± 5.8	-0.71 ± 1.2
L	0.21 ± 0.1	-0.84 ± 0.2	-0.84 ± 0.3	30.36 ± 3.5	-0.13 ± 1.0

[a]No significant treatment effects (P > 0.05)

Table 7. Change (final minus initial concentrations) in mean creatinine, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), and creatine kinase (CK) concentrations (± SE) for the water (water access = Yes, no water access = No) and density (high = H, medium = M, low = L) treatments for all six shipments

Water/Density Treatments [a]	Creatinine (mg/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)
Yes	0.13 ± 0.0	1.31 ± 0.1	-0.03 ± 0.0	10.91 ± 2.9	68.10 ± 80.2
No	0.15 ± 0.1	1.48 ± 0.2	-0.03 ± 0.0	13.47 ± 3.7	42.63 ± 159.4
H	0.19 ± 0.1	1.63 ± 0.1	-0.03 ± 0.0	15.43 ± 3.8	243.6 ± 184.9
M	0.12 ± 0.1	1.34 ± 0.1	-0.02 ± 0.0	12.83 ± 2.7	-39.3 ± 92.6
L	0.10 ± 0.0	1.14 ± 0.1	-0.05 ± 0.0	7.02 ± 4.7	-25.5 ± 28.9

[a]No significant treatment effects (P > 0.05)

Table 8. Change (final minus initial concentrations) in mean aspartate aminotransferase (AST), globulins, albumin/globulin (A/G) ratio, and gamma glutamyl transferase (GGT) concentrations (± SE) for the water (water access = Yes, no water access = No) and density (high = H, medium = M, low = L) treatments for all six shipments

Water/Density Treatments	AST (U/l)	Globulins (g/dL)	A/G Ratio	GGT (U/l)
Yes	28.86 ± 6.58	0.19 ± 0.0	0.0095 ± 0.0	0.30 ± 0.4
No	29.40 ± 5.6	0.31 ± 0.1	0.0027 ± 0.0	0.67 ± 0.8
H	42.93 ± 7.4[a]	0.27 ± 0.1	0.012 ± 0.0	0.77 ± 0.6
M	31.77 ± 5.7[a]	0.23 ± 0.0	0.011 ± 0.0	0.65 ± 0.6
L	12.43 ± 6.3[b]	0.18 ± 0.1	-0.001 ± 0.0	-0.15 ± 0.6

[a,b]Means for each treatment within a column with different superscripts differ ($P < 0.05$)

DISCUSSION

Whiting (1999) conducted an observational study from 1995 to 1997 on the densities of 296 loads of loose transported horses from various locations, including auctions and slaughter plants. In that study, Whiting (1999) compared the actual density onboard each trailer to those recommended by the Canadian loading density standards in the Canadian Code of Practice published in 1998. The majority of loads were at or below the maximum Canadian recommended density standards (Whiting, 1999). The high density utilized in the current study was close to the limits recommended by the Canadian Code of Practice (CARC, 2001). Maximum density requirements did not change from the 1998 to the 2001 version of the Canadian Code of Practice. The high densities used in the current study were likely reasonable representations of the common densities used in commercial transport.

It has been debated as to whether it is better to have horses transported in high vs. low densities in terms of levels of aggression (Collins et al., 2000; Friend, 2000; Friend, 2001; Stull, 2001; Stull and Rodiek, 2002). The high density may help prevent severe injuries from kicking since there likely would not be a lot of room to direct an aggressive kick. If a horse were to kick as a reaction to being bitten, it likely would kick the wrong horse in response. However, the high density would prevent horses that were being bitten from escaping (Collins et al., 2000; Friend, 2001). The arguments for the low density were that the less dominant horses could get away from aggressive horses (Collins et al., 2000; Friend, 2001), but the low density allowed room for full contact kicking. Noticeably less banging occurs on trailers in high density groups (Friend, 2001), which could indicate that horses in the high density compartment are not moving around as much.

Based on observations from our video, it appeared that the amount of aggression during a shipment was dependent on individual horses and not density. There were usually one to two consistently aggressive horses in each compartment for the duration of the trip. Grandin (1999) noted that it may be just a few aggressive horses that cause most injuries during transportation. Due to the cameras being mounted above the horses, it was not possible to accurately record kicking behavior. When biting behavior was observed, only a few horses in the high density group that were picked on were able to escape the aggressor. If an aggressive horse was in the low density compartment, it often took up a large portion of the compartment and defended the space from other horses. This lead to the less dominant horses pressed up against the sides of the trailer opposite the aggressive horse in order to avoid being bitten. As a result, with the exception of the one or two dominant horses, the horses in the low density compartment were often in a density similar to those in the high density group.

Although the bouts of aggression were similar for all compartments, the horses in the low density treatment may have been able to cope better with the aggressive horses since they were likely able to avoid aggressive individuals. Horses in the higher density probably had a harder time escaping aggressive encounters due to limited space. Consequently, the horses in the higher density may have been approaching learned helplessness and(or) experienced increased levels of stress due to their likely inability to escape. The conditions the horses experienced in the high density may be comparable to dogs subjected to escapable vs. inescapable shock. Seligman and Maier (1967) conducted a study in which dogs were paired so that one dog could terminate the shock at will and the other dog received the shock, but had no control over it. When the dogs were put in a box with an escape route from the shock, the dog that previously terminated the shock at will, promptly escaped to avoid shock whereas the dog that received the shock at random appeared to passively accept the shocks. A similar study on escapable vs. inescapable shock conducted with rats (Weiss, 1972) demonstrated that rats that had no control over receiving the shocks showed more signs of stress as compared to rats that were able to escape from the shocks. Inescapable rats had significantly more gastric ulcers and lost more weight than rats able to avoid the shock. If horses were unable to escape aggressive individuals onboard the trailer, it seems likely that they would develop similar signs of stress as found in Weiss (1972).

In order to determine if any horses in this study were approaching learned helplessness, the data would have to be further analyzed to determine escape response in different density treatments. A horse in high density conditions would first have to be observed to not be able to escape from an aggressor despite multiple aggressive encounters. Once the horse was observed to not escape the aggressor, it would need to be put in a lower density treatment where it could potentially escape. If at that time, the horse still did not move to escape the aggressor, than it would be likely that the horse had developed learned helplessness.

Some important issues still need to be considered when determining optimal density to minimize stress. In this study, two horses lay down during transport and both horses were in the high density treatments. The first horse that went down was found dead upon arrival of the slaughter plant. No video data were available on that horse since the video equipment malfunctioned. However, the other horse that went down was recorded on the multiplexers. When the horse went down, it laid on its side with its legs straight out. The other horses initially made room for the downer horse for about the first hour and then proceeded to eventually stand over the horse on the floor. The horse remained on the floor for about 3 h until the truck driver coaxed it to stand again when the load was checked. Despite not finding any physiological differences between density groups, it seems likely that some factor in the high density, and not the medium or low densities, stresses certain horses to the point of lying down. Another issue is that the high density may not allow many horses to access water bowls if the watering system is utilized. In this study, several of the video cameras malfunctioned so it was not possible to obtain accurate behavioral data on access to the water bowls at different densities.

One major consideration in this study is that the shipments may not have been transported long enough to see any differences in dehydration and physiological signs of stress for the different treatments. Shipments closer to the maximum travel time may show more signs of stress and be more likely to utilize the watering system. However, based on the duration, temperature, and road conditions of these shipments, it appears the maximum density used in this study could be used in the industry without causing excessive stress on the horses and also to maximize efficiency for the horse owners.

It was originally hypothesized that more aggression would be occurring at the beginning of the shipment as compared to the end. It seemed likely that horses would work out a pecking order at the beginning and(or) become fatigued towards the end of transport. However, the aggression in this study did not significantly differ from first half to the second half. Like the density and dehydration treatment effects, the shipment probably was not long enough for horses to become fatigued to the point of reduced aggressive encounters. Friend (2000) transported horses commercially and determined that most interactive behavior between non-watered horses stopped after 24 h and by 27 h, the horses were significantly quieter. Horses in that study that were provided with water went 30 h before showing severe signs of fatigue. Similarly, Stull (1999) determined that as the duration of transport increased, especially at 27 or more hours, the horses became notably fatigued. Therefore, based on the aggression data and conditions of transport in this study, the hypothesis that more aggression would occur at the beginning of the shipment as compared to the end should be rejected.

Some of the horses did not have blood constituents within the normal range, as determined by the Texas A&M Veterinary Medical Diagnostic Laboratory. Horses were in various conditions of health so it seems reasonable to expect a few horses to show physiological signs of being ill as was the case in this study. However, most blood constituents were within the normal range and all horses appeared to be fit for transport prior to loading.

Horses generally did not show physiological signs of stress indicative of the density or water treatments. Change in mean cortisol, aldosterone, electrolytes and the total serum protein did not differ in any of the treatments. Any signs of stress in this study due to aggression, density and water treatments could have been masked by stress caused by restraint for blood collection and weighing before and after transport.

The only significant difference for the physiological data was AST being lower in the low density treatment as compared to the high and medium densities. As a measure of liver function, one would not expect AST to provide very meaningful information for this study. Likely, horses had AST concentrations that reflected a poor state of health prior to transportation. Due to the large numbers of comparisons, it may also be a matter of chance that AST was different in the low density compartments.

In the preliminary study conducted by this lab (Iacono et al., 2005), horses with water access lost less weight than the non-watered control horses during hot weather transport. Mean temperatures of the preliminary study and the current study were determined by two to four temperature data loggers mounted inside the trailer that took readings every 5 min. These 5-min readings were averaged together and then averaged with the other temperature data loggers to determine a mean temperature for the duration of a shipment. The mean temperature for the shipment where horses lost less weight when given water as compared to the controls in the preliminary study was 30˚C. The last three shipments in the current study also had a combined mean temperature of 30˚C. Despite having the same mean temperature as the shipment transported in hot weather in the preliminary study, the current study determined that water availability did not prevent dehydration in the horses as determined by similar changes in weight loss or concentrations of total serum protein and electrolytes in the watered and non-watered treatments for the last three shipments. Also, the first three shipments in this study had a mean temperature that was cooler than the last three shipments (22˚C vs. 30˚C). When the first three shipments were compared with the last three shipments, no differences in change in weight loss, serum chemistry profile, or electrolyte concentrations occurred. Likewise, when only the last three hot shipments were examined for treatment effects no differences were observed for the density treatments, but a slight trend was evident for the water treatment. Based on these results, it seems likely that hot weather was not the only factor contributing to water consumption and weight loss in the preliminary study. Other possible factors leading to water consumption need to be examined to determine when, if at all, it would be worthwhile to provide water to horses during transport in similar conditions.

Because behavioral data were not available for the watering sessions, it was difficult to determine to what extent the horses utilized the watering system. It was not known how many or which individual horses drank onboard the trailer during the watering sessions. In the preliminary study by Iacono et al. (2005), the densities for horses of similar weight to those in the current study were less (205 kg/m^2 to 318 kg/m^2) so most horses likely had easy access to the water bowls. The majority of the horses in the preliminary study were observed to have access to at least one water bowl during the watering sessions. In the current study, several horses may not have been able to access the water bowls due to higher density levels and possibly aggressive horses. It would have been helpful to draw blood from all the horses due to the likely variation in amount of water consumed per horse. However, based on the weight loss data taken from all the horses, it appears that the utilization of the watering system was limited in this study.

CONCLUSION

Density and water access did not substantially affect aggression, dehydration, physiological indications of stress, and weight loss. Therefore, the hypothesis that horses would have fewer aggressive encounters and be less stressed if transported in a medium or low density compartment should be rejected based on the behavior, physiology, and weight loss data from this study. Individual horses appeared to influence the level of aggression in each compartment. The amount of aggression was the same for the first and second half of the shipments, indicating that contrary to the original hypothesis, 20 h is not long enough for horses to become significantly fatigued to the point of not being aggressive. The physiological signs of dehydration, along with weight loss, did not indicate that access to water was useful for the horses. The hypothesis that horses with water access would lose less weight and show fewer signs of dehydration and stress compared to non-watered controls should be rejected based on the behavior, physiology, and weight loss data from this study. Shipments of longer duration may show significant differences in aggression, dehydration, physiological measures of stress, and weight loss for different density and water treatments.

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APPENDIX A

Table A1. Serum protein (S. Protein), albumin, calcium (Ca), and phosphorus (P) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment one

Horse	Density	Water	Sample	S. Protein (g/dL)	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)
1	M	Yes	1	7.1	3.0	10.1	5.4
1	M	Yes	2	7.6	3.3	9.1	4.6
2	L	No	1	3.6	1.6	8.1	2.2
2	L	No	2	4.0	1.8	8.0	2.2
3	M	Yes	1	6.3	3.1	11.4	2.5
3	M	Yes	2	7.3	3.5	11.7	2.4
4	L	No	1	7.1	3.1	10.7	3.5
4	L	No	2	7.8	3.4	10.6	2.6
5	M	Yes	1	6.6	2.7	10.7	3.1
5	M	Yes	2	7.3	3.0	9.3	2.7
6	M	Yes	1	7.5	3.2	11.8	2.7
6	M	Yes	2	8.1	3.5	11.1	2.6
7	H	Yes	1	5.1	2.5	8.9	3.2
7	H	Yes	2	7.3	3.7	10.8	4.6
8	H	Yes	1	6.0	2.5	8.6	2.2
8	H	Yes	2	7.8	3.3	10.1	2.4
9	M	Yes	1	7.1	2.9	11.4	3.6
9	M	Yes	2	7.3	3.0	11.1	2.3
10	L	No	1	6.9	2.7	10.9	2.5
10	L	No	2	7.4	2.9	11.5	2.2
11	H	Yes	1	7.5	3.5	11.1	4.4
11	H	Yes	2	5.9	2.8	8.8	2.7
12	H	Yes	1	7.3	2.9	11.1	2.5
12	H	Yes	2	7.7	3.2	10.7	2.9
13	L	No	1	6.7	2.7	11.4	2.8
13	L	No	2	7.8	3.1	10.8	3.2
14	H	Yes	1	7.4	2.9	11.2	3.3
14	H	Yes	2	7.6	3.0	10.6	3.4
15	L	No	1	7.7	3.0	10.3	4.4
15	L	No	2	8.4	3.3	9.2	1.8

Table A2. Glucose, blood urea nitrogen (BUN), creatinine, and total bilirubin concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment one

Horse	Density	Water	Sample	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)
1	M	Yes	1	106	17.8	1.5	2.1
1	M	Yes	2	155	20.0	1.5	3.3
2	L	No	1	81	15.3	1.4	1.2
2	L	No	2	114	19.1	1.3	2.2
3	M	Yes	1	92	14.7	1.9	1.5
3	M	Yes	2	125	17.0	1.7	3.7
4	L	No	1	93	10.8	1.3	1.6
4	L	No	2	127	17.8	1.3	2.6
5	M	Yes	1	87	9.5	1.6	1.0
5	M	Yes	2	138	13.6	1.6	2.6
6	M	Yes	1	90	20.0	1.7	1.0
6	M	Yes	2	120	21.9	1.8	2.6
7	H	Yes	1	92	26.1	1.5	1.7
7	H	Yes	2	130	28.1	1.6	3.1
8	H	Yes	1	96	13.2	1.0	2.2
8	H	Yes	2	154	23.6	1.2	3.9
9	M	Yes	1	95	11.1	1.4	1.5
9	M	Yes	2	134	16.6	1.3	3.1
10	L	No	1	93	12.6	1.7	1.5
10	L	No	2	154	18.5	1.5	2.5
11	H	Yes	1	91	16.0	1.0	1.9
11	H	Yes	2	160	17.6	1.0	2.2
12	H	Yes	1	108	20.8	1.8	2.7
12	H	Yes	2	169	21.8	1.9	5.3
13	L	No	1	75	20.1	0.8	1.0
13	L	No	2	113	19.9	0.7	2.4
14	H	Yes	1	100	26.2	1.4	1.1
14	H	Yes	2	133	26.8	1.4	2.0
15	L	No	1	93	22.8	1.2	0.9
15	L	No	2	144	25.9	1.4	1.8

Table A3. Direct bilirubin, alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment one

Horse	Density	Water	Sample	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)	AST (U/l)
1	M	Yes	1	0.2	216	575	327
1	M	Yes	2	0.2	221	1168	407
2	L	No	1	0.1	109	212	149
2	L	No	2	0.1	114	129	163
3	M	Yes	1	0.2	135	363	247
3	M	Yes	2	0.1	160	386	301
4	L	No	1	0.2	173	141	220
4	L	No	2	0.2	187	172	246
5	M	Yes	1	0.3	165	209	192
5	M	Yes	2	0.1	181	189	232
6	M	Yes	1	0.2	140	348	231
6	M	Yes	2	0.2	158	227	257
7	H	Yes	1	0.1	68	414	188
7	H	Yes	2	0.2	106	1028	313
8	H	Yes	1	0.2	187	1957	427
8	H	Yes	2	0.1	229	5923	674
9	M	Yes	1	0.2	165	249	284
9	M	Yes	2	0.2	176	350	307
10	L	No	1	0.2	134	221	186
10	L	No	2	0.1	150	185	201
11	H	Yes	1	0.2	283	325	263
11	H	Yes	2	0.1	205	194	200
12	H	Yes	1	0.2	96	292	244
12	H	Yes	2	0.1	119	266	278
13	L	No	1	0.1	188	175	186
13	L	No	2	0.1	217	535	253
14	H	Yes	1	0.2	257	371	246
14	H	Yes	2	0.1	262	435	274
15	L	No	1	0.3	173	334	262
15	L	No	2	0.1	204	540	330

Table A4. Globulins, albumin/globulin (A/G) ratio, gamma glutamyl transferase (GGT), and cortisol concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment one

Horse	Density	Water	Sample	Globulins (g/dL)	A/G Ratio	GGT (U/l)	Cortisol (ng/mL))
1	M	Yes	1	4.1	0.73	16	125.5
1	M	Yes	2	4.3	0.77	17	----
2	L	No	1	2.0	0.80	7	113.7
2	L	No	2	2.2	0.82	5	----
3	M	Yes	1	3.2	0.97	10	54.2
3	M	Yes	2	3.8	0.92	12	79.5
4	L	No	1	4.0	0.77	10	35.6
4	L	No	2	4.4	0.77	10	36.1
5	M	Yes	1	3.9	0.69	9	65.3
5	M	Yes	2	4.3	0.70	10	42.0
6	M	Yes	1	4.3	0.74	8	28.5
6	M	Yes	2	4.6	0.76	7	52.9
7	H	Yes	1	2.6	0.96	9	135.6
7	H	Yes	2	3.6	1.03	13	52.9
8	H	Yes	1	3.5	0.71	10	69.9
8	H	Yes	2	4.5	0.73	11	88.3
9	M	Yes	1	4.2	0.69	16	53.3
9	M	Yes	2	4.3	0.7	17	98.5
10	L	No	1	4.2	0.64	10	50.7
10	L	No	2	4.5	0.64	9	32.0
11	H	Yes	1	4.0	0.88	16	131.7
11	H	Yes	2	3.1	0.90	10	38.5
12	H	Yes	1	4.4	0.66	4	58.8
12	H	Yes	2	4.5	0.71	4	53.7
13	L	No	1	4.0	0.68	6	28.4
13	L	No	2	4.7	0.66	8	100.3
14	H	Yes	1	4.5	0.64	12	79.7
14	H	Yes	2	4.6	0.65	11	30.4
15	L	No	1	4.7	0.64	12	50.6
15	L	No	2	5.1	0.65	11	55.4

Table A5. Electrolyte and aldosterone concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment one

Horse	Density	Water	Sample	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)
1	M	Yes	1	143	3.4	105	33.7
1	M	Yes	2	----	----	----	80.4
2	L	No	1	139	3.6	111	106.6
2	L	No	2	141	4.8	118	62.4
3	M	Yes	1	143	4.0	110	25.9
3	M	Yes	2	141	4.7	111	275.0
4	L	No	1	143	3.3	107	111.6
4	L	No	2	142	4.0	112	66.5
5	M	Yes	1	145	3.7	107	38.9
5	M	Yes	2	144	4.6	111	7.3
6	M	Yes	1	142	3.6	106	17.0
6	M	Yes	2	145	5.8	114	30.1
7	H	Yes	1	139	3.3	109	19.3
7	H	Yes	2	143	3.6	110	75.7
8	H	Yes	1	126	3.2	95	35.1
8	H	Yes	2	142	4.2	110	78.0
9	M	Yes	1	143	4.3	108	28.1
9	M	Yes	2	144	4.4	114	----
10	L	No	1	144	3.5	109	55.5
10	L	No	2	147	4.8	116	20.7
11	H	Yes	1	142	4.1	108	119.6
11	H	Yes	2	139	5.8	112	37.8
12	H	Yes	1	141	3.6	107	4.7
12	H	Yes	2	----	----	----	73.3
13	L	No	1	144	3.6	100	92.1
13	L	No	2	139	4.7	106	42.0
14	H	Yes	1	144	3.7	107	45.1
14	H	Yes	2	142	4.3	108	47.8
15	L	No	1	145	3.4	107	13.2
15	L	No	2	143	4.8	108	113.2

Table A6. Serum protein, albumin, calcium (Ca), and phosphorus (P) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment two

Horse	Density	Water	Sample	Serum Protein (g/dL)	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)
1	M	No	1	6.8	3.1	10.6	3.8
1	M	No	2	7.2	3.4	10.5	4.0
2	H	Yes	1	6.4	2.8	10.0	1.8
2	H	Yes	2	7.4	3.2	11.3	2.5
3	L	Yes	1	7.7	3.0	10.1	2.8
3	L	Yes	2	5.0	1.9	7.7	2.7
4	H	Yes	1	7.5	3.2	10.9	4.8
4	H	Yes	2	7.9	3.4	9.2	3.1
5	L	Yes	1	8.4	3.2	10.6	1.3
5	L	Yes	2	8.8	3.3	11.0	2.4
6	M	No	1	7.0	3.0	11.1	4.1
6	M	No	2	7.9	3.4	11.0	3.3
7	M	No	1	6.8	2.8	11.0	3.8
7	M	No	2	7.5	3.2	10.5	4.7
8	H	Yes	1	7.6	3.1	10.5	1.6
8	H	Yes	2	7.7	3.1	10.1	1.7
9	H	Yes	1	8.2	2.8	10.4	2.7
9	H	Yes	2	8.2	2.9	10.4	3.4
10	H	Yes	1	8.2	2.7	10.9	2.7
10	H	Yes	2	7.7	2.7	10.7	4.5
11	M	No	1	7.8	3.0	10.6	2.4
11	M	No	2	7.5	2.6	10.9	3.7
12	L	Yes	1	8.4	2.8	9.9	1.8
12	L	Yes	2	8.2	2.8	9.6	2.7
13	L	Yes	1	8.1	3.4	11.5	2.4
13	L	Yes	2	8.3	3.5	10.6	3.7
14	M	No	1	8.9	3.0	10.7	3.7
14	M	No	2	9.0	2.9	9.9	5.7
15	L	Yes	1	7.8	2.9	10.3	4.2
15	L	Yes	2	8.6	3.3	9.7	3.6

Table A7. Glucose, blood urea nitrogen (BUN), creatinine, and total bilirubin concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment two

Horse	Density	Water	Sample	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)
1	M	No	1	102	17.7	0.7	0.6
1	M	No	2	147	14.6	1.0	2.0
2	H	Yes	1	89	17.0	1.3	3.9
2	H	Yes	2	109	16.2	1.4	6.0
3	L	Yes	1	104	22.0	0.9	4.4
3	L	Yes	2	75	17.6	0.8	3.1
4	H	Yes	1	107	21.4	0.9	0.9
4	H	Yes	2	121	19.1	1.2	3.1
5	L	Yes	1	147	14.0	0.8	2.7
5	L	Yes	2	175	13.3	0.8	4.2
6	M	No	1	123	20.9	0.8	0.5
6	M	No	2	127	20.5	1.2	1.6
7	M	No	1	109	20.7	0.9	1.0
7	M	No	2	127	21.0	1.1	2.1
8	H	Yes	1	181	14.8	0.9	4.1
8	H	Yes	2	148	11.8	0.8	4.6
9	H	Yes	1	125	18.2	1.4	2.2
9	H	Yes	2	142	16.9	1.5	2.7
10	H	Yes	1	120	22.2	1.2	1.5
10	H	Yes	2	119	18.5	1.2	2.3
11	M	No	1	118	19.7	0.9	1.3
11	M	No	2	96	19.2	1.1	2.1
12	L	Yes	1	104	19.8	0.6	0.6
12	L	Yes	2	158	14.2	0.7	1.8
13	L	Yes	1	103	23.2	1.2	0.9
13	L	Yes	2	157	22.8	1.3	2.4
14	M	No	1	126	15.0	0.6	1.4
14	M	No	2	126	12.5	0.6	2.2
15	L	Yes	1	110	21.2	0.9	0.9
15	L	Yes	2	151	20.6	1.1	1.8

Table A8. Direct bilirubin, alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment two

Horse	Density	Water	Sample	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)	AST (U/l)
1	M	No	1	0.2	126	144	271
1	M	No	2	0.1	135	198	322
2	H	Yes	1	0.1	76	307	405
2	H	Yes	2	0.2	87	448	503
3	L	Yes	1	0.2	179	203	252
3	L	Yes	2	0.1	110	213	152
4	H	Yes	1	0.2	156	468	315
4	H	Yes	2	0.1	162	----	361
5	L	Yes	1	0.1	131	167	469
5	L	Yes	2	0.2	156	176	449
6	M	No	1	0.1	95	210	215
6	M	No	2	0.2	116	336	289
7	M	No	1	0.1	189	3158	636
7	M	No	2	0.1	211	924	651
8	H	Yes	1	0.1	101	161	209
8	H	Yes	2	0.1	155	359	263
9	H	Yes	1	0.1	228	242	242
9	H	Yes	2	0.1	229	230	259
10	H	Yes	1	0.1	158	177	231
10	H	Yes	2	0.1	150	169	229
11	M	No	1	0.1	104	93	159
11	M	No	2	0.1	101	79	165
12	L	Yes	1	0.1	232	493	222
12	L	Yes	2	0.1	235	234	231
13	L	Yes	1	0.2	203	543	435
13	L	Yes	2	0.1	209	318	458
14	M	No	1	0.1	243	397	226
14	M	No	2	0.1	225	353	231
15	L	Yes	1	0.3	146	182	281
15	L	Yes	2	0.1	152	223	325

Table A9. Globulins, albumin/globulin (A/G) ratio, gamma glutamyl transferase (GGT), and cortisol concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment two

Horse	Density	Water	Sample	Globulins (g/dL)	A/G Ratio	GGT (U/l)	Cortisol (ng/mL)
1	M	No	1	3.7	0.84	14	41.1
1	M	No	2	3.8	0.89	16	58.3
2	H	Yes	1	3.6	0.78	7	56.2
2	N	Yes	2	4.2	0.76	10	145.1
3	L	Yes	1	4.7	0.64	9	34.8
3	L	Yes	2	3.1	0.61	7	86.4
4	H	Yes	1	4.3	0.74	8	47.9
4	H	Yes	2	4.5	0.76	13	68.7
5	L	Yes	1	5.2	0.62	21	142.7
5	L	Yes	2	5.5	0.60	23	26.8
6	M	No	1	4.0	0.75	18	41.8
6	M	No	2	4.5	0.76	22	46.8
7	M	No	1	4.0	0.70	47	68.5
7	M	No	2	4.3	0.74	54	152.4
8	H	Yes	1	4.5	0.69	8	67.3
8	H	Yes	2	4.6	0.67	8	60.8
9	H	Yes	1	5.4	0.52	11	25.5
9	H	Yes	2	5.3	0.55	10	33.0
10	H	Yes	1	5.5	0.49	10	121.3
10	H	Yes	2	5.0	0.54	11	52.2
11	M	No	1	4.8	0.63	7	75.3
11	M	No	2	4.9	0.53	7	54.7
12	L	Yes	1	5.6	0.50	9	136.7
12	L	Yes	2	5.4	0.52	11	152.6
13	L	Yes	1	4.7	0.72	21	147.3
13	L	Yes	2	4.8	0.73	22	151.3
14	M	No	1	5.9	0.51	7	142.6
14	M	No	2	6.1	0.48	7	73.8
15	L	Yes	1	4.9	0.59	11	108.7
15	L	Yes	2	5.3	0.62	15	101.8

Table A10. Electrolyte and aldosterone concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment two

Horse	Density	Water	Sample	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)
1	M	No	1	141	4.0	105	89.9
1	M	No	2	139	4.2	107	11.8
2	H	Yes	1	141	3.7	108	4.6
2	H	Yes	2	144	3.8	109	18.5
3	L	Yes	1	139	3.6	104	37.9
3	L	Yes	2	117	3.9	93	30.6
4	H	Yes	1	144	4.7	103	15.3
4	H	Yes	2	137	3.9	104	7.2
5	L	Yes	1	134	4.9	100	23.7
5	L	Yes	2	134	4.0	102	219.5
6	M	No	1	141	4.3	103	6.3
6	M	No	2	140	5.3	107	80.0
7	M	No	1	135	4.9	102	20.9
7	M	No	2	135	5.4	104	40.3
8	H	Yes	1	131	2.8	99	23.7
8	H	Yes	2	137	2.6	100	95.8
9	H	Yes	1	141	3.7	103	317.5
9	H	Yes	2	146	3.5	107	46.5
10	H	Yes	1	141	5.7	105	12.8
10	H	Yes	2	137	4.4	106	58.7
11	M	No	1	141	6.6	107	17.6
11	M	No	2	138	5.9	106	25.7
12	L	Yes	1	138	4.1	104	16.8
12	L	Yes	2	137	3.7	108	14.2
13	L	Yes	1	143	5.7	100	1.8
13	L	Yes	2	138	4.8	105	6.8
14	M	No	1	134	4.5	99	----
14	M	No	2	137	4.1	101	19.2
15	L	Yes	1	144	3.5	104	4.0
15	L	Yes	2	137	3.7	106	19.1

Table A11. Serum protein, albumin, calcium (Ca), and phosphorus (P) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment three

Horse	Density	Water	Sample	Serum Protein (g/dL)	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)
1	H	No	1	7.1	3.3	11.3	3.3
1	H	No	2	7.5	3.5	9.6	3.4
2	H	No	1	7.8	3.4	11.3	3.1
2	H	No	2	8.4	3.6	10.3	3.4
3	M	Yes	1	6.8	2.9	11.1	2.5
3	M	Yes	2	7.3	3.2	10.9	2.3
4	H	No	1	8.6	3.0	11.5	2.7
4	H	No	2	8.7	3.1	11.1	4.3
5	M	Yes	1	7.3	3.0	11.6	3.1
5	M	Yes	2	7.4	3.1	10.4	2.3
6	L	Yes	1	7.2	2.9	11.3	2.1
6	L	Yes	2	7.9	3.1	10.9	2.5
7	H	No	1	6.9	2.9	11.2	4.6
7	H	No	2	7.2	3.0	10.4	2.9
8	H	No	1	7.3	2.8	11.1	3.4
8	H	No	2	7.8	3.0	10.8	2.4
9	L	Yes	1	7.9	3.2	11.5	4.0
9	L	Yes	2	8.4	3.4	10.3	2.5
10	M	Yes	1	7.6	2.9	11.0	2.4
10	M	Yes	2	8.2	3.1	10.3	2.2
11	L	Yes	1	6.8	3.0	9.9	3.2
11	L	Yes	2	7.6	3.3	9.0	4.6
12	L	Yes	1	7.3	3.0	10.1	1.2
12	L	Yes	2	7.4	2.9	10.2	1.4
13	M	Yes	1	----	----	----	----
13	M	Yes	2	----	----	----	----

Table A12. Glucose, blood urea nitrogen (BUN), creatinine, and total bilirubin concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment three

Horse	Density	Water	Sample	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)
1	H	No	1	90	26.7	1.0	0.7
1	H	No	2	124	25.4	1.2	1.6
2	H	No	1	98	22.3	1.1	2.7
2	H	No	2	176	22.5	1.0	6.1
3	M	Yes	1	87	19.8	0.9	1.2
3	M	Yes	2	110	16.8	1.0	3.1
4	H	No	1	89	16.9	0.8	0.8
4	H	No	2	111	13.7	0.9	2.1
5	M	Yes	1	86	19.5	1.1	1.0
5	M	Yes	2	142	15.5	1.2	2.1
6	L	Yes	1	87	23.4	0.8	0.9
6	L	Yes	2	120	20.3	0.9	2.4
7	H	No	1	92	21.4	0.8	1.0
7	H	No	2	175	16.0	0.9	2.6
8	H	No	1	98	19.9	1.1	1.3
8	H	No	2	164	16.6	1.0	2.7
9	L	Yes	1	93	16.2	0.8	1.3
9	L	Yes	2	160	13.9	0.8	3.4
10	M	Yes	1	106	23.9	0.9	0.8
10	M	Yes	2	156	20.4	1.1	2.5
11	L	Yes	1	102	16.7	0.8	0.8
11	L	Yes	2	134	16.4	1.0	1.6
12	L	Yes	1	99	10.2	1.1	4.9
12	L	Yes	2	110	8.9	1.1	6.1
13	M	Yes	1	----	----	----	----
13	M	Yes	2	----	----	----	----

Table A13. Direct bilirubin, alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment three

Horse	Density	Water	Sample	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)	AST (U/l)
1	H	No	1	0.1	220	179	290
1	H	No	2	0.1	230	270	336
2	H	No	1	0.1	222	300	287
2	H	No	2	0.1	221	214	308
3	M	Yes	1	0.2	159	197	228
3	M	Yes	2	0.1	174	616	300
4	H	No	1	0.2	297	156	235
4	H	No	2	0.1	294	181	239
5	M	Yes	1	0.2	186	191	222
5	M	Yes	2	0.1	180	351	266
6	L	Yes	1	0.2	98	505	295
6	L	Yes	2	0.1	102	441	342
7	H	No	1	0.2	181	233	286
7	H	No	2	0.1	189	378	309
8	H	No	1	0.1	162	231	196
8	H	No	2	0.1	176	221	218
9	L	Yes	1	0.2	143	273	496
9	L	Yes	2	0.2	134	383	512
10	M	Yes	1	0.1	211	286	393
10	M	Yes	2	0.1	241	314	433
11	L	Yes	1	0.2	135	288	1493
11	L	Yes	2	0.1	150	253	1483
12	L	Yes	1	0.1	83	138	410
12	L	Yes	2	0.1	95	160	412
13	M	Yes	1	----	----	----	----
13	M	Yes	2	----	----	----	----

Table A14. Globulins, albumin/globulin (A/G) ratio, gamma glutamyl transferase (GGT), and cortisol concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment three

Horse	Density	Water	Sample	Globulins (g/dL)	A/G Ratio	GGT (U/l)	Cortisol (ng/mL)
1	H	No	1	3.8	0.87	14	101.0
1	H	No	2	4.0	0.88	17	88.4
2	H	No	1	4.4	0.77	16	64.3
2	H	No	2	4.8	0.75	14	114.5
3	M	Yes	1	3.9	0.74	8	53.8
3	M	Yes	2	4.1	0.78	8	87.3
4	H	No	1	5.6	0.54	9	75.5
4	H	No	2	5.6	0.55	9	38.6
5	M	Yes	1	4.3	0.70	11	27.7
5	M	Yes	2	4.3	0.72	11	124.2
6	L	Yes	1	4.3	0.67	8	123.4
6	L	Yes	2	4.8	0.65	9	92.7
7	H	No	1	4.0	0.73	15	119.5
7	H	No	2	4.2	0.71	17	69.3
8	H	No	1	4.5	0.62	10	49.2
8	H	No	2	4.8	0.63	12	91.2
9	L	Yes	1	4.7	0.68	14	109.1
9	L	Yes	2	5.0	0.68	14	88.7
10	M	Yes	1	4.7	0.62	19	102.5
10	M	Yes	2	5.1	0.61	21	150.7
11	L	Yes	1	3.8	0.79	16	34.4
11	L	Yes	2	4.3	0.77	17	157.2
12	L	Yes	1	4.3	0.70	12	64.8
12	L	Yes	2	4.5	0.64	12	94.6
13	M	Yes	1	----	----	----	87.1
13	M	Yes	2	----	----	----	86.2

Table A15. Electrolyte and aldosterone concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment three

Horse	Density	Water	Sample	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)
1	H	No	1	141	7.1	106	26.8
1	H	No	2	144	4.7	111	30.7
2	H	No	1	137	9.7	107	53.3
2	H	No	2	143	4.8	110	11.0
3	M	Yes	1	141	6.9	105	57.6
3	M	Yes	2	140	4.8	106	33.4
4	H	No	1	139	6.3	104	9.0
4	H	No	2	140	4.1	104	29.9
5	M	Yes	1	139	9.8	108	38.2
5	M	Yes	2	142	4.8	108	16.1
6	L	Yes	1	140	6.0	104	27.2
6	L	Yes	2	146	4.7	112	33.3
7	H	No	1	142	5.4	106	3.6
7	H	No	2	141	3.5	107	113.0
8	H	No	1	139	6.6	105	6.8
8	H	No	2	139	4.3	105	18.1
9	L	Yes	1	139	7.8	108	7.8
9	L	Yes	2	141	5.4	109	58.8
10	M	Yes	1	139	8.2	105	37.0
10	M	Yes	2	145	4.6	111	32.3
11	L	Yes	1	137	5.5	101	122.5
11	L	Yes	2	143	4.6	105	26.7
12	L	Yes	1	131	5.3	100	42.5
12	L	Yes	2	131	3.5	98	207.8
13	M	Yes	1	141	8.0	108	125.6
13	M	Yes	2	146	5.6	114	50.6

Table A16. Serum protein, albumin, calcium (Ca), and phosphorus (P) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment four

Horse	Density	Water	Sample	Serum Protein (g/dL)	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)
1	H	No	1	7.7	2.3	9.8	2.1
1	H	No	2	9.4	2.8	8.3	4.6
2	M	Yes	1	7.0	3.0	10.7	2.8
2	M	Yes	2	7.7	3.3	10.0	4.3
3	M	Yes	1	7.8	3.0	11.1	2.7
3	M	Yes	2	8.5	3.2	10.7	3.5
4	H	No	1	7.0	3.0	11.1	3.0
4	H	No	2	8.1	3.4	9.9	3.0
5	H	No	1	6.8	3.0	11.2	3.5
5	H	No	2	7.7	3.3	10.3	5.4
6	H	No	1	6.9	3.2	11.4	2.6
6	H	No	2	7.4	3.3	9.6	5.0
7	H	No	1	6.7	2.8	10.4	2.3
7	H	No	2	7.8	3.3	10.6	3.2
8	L	Yes	1	8.2	2.6	9.9	3.6
8	L	Yes	2	9.1	2.9	10.1	4.9
9	M	Yes	1	8.1	2.5	10.9	3.0
9	M	Yes	2	8.8	2.9	10.3	3.9
10	L	Yes	1	6.9	3.1	11.0	4.5
10	L	Yes	2	7.0	3.2	9.0	6.5
11	L	Yes	1	8.9	2.9	11.2	3.5
11	L	Yes	2	8.8	2.9	10.4	4.8
12	M	Yes	1	7.7	2.8	10.9	2.8
12	M	Yes	2	7.7	2.7	10.2	3.3
13	L	Yes	1	8.0	2.8	12.1	2.7
13	L	Yes	2	8.3	3.0	10.6	3.4
14	L	Yes	1	9.1	2.0	16.2	2.2
14	L	Yes	2	9.6	2.1	11.6	2.2
15	M	Yes	1	7.7	3.4	11.2	3.4
15	M	Yes	2	8.1	3.6	9.5	4.0

Table A17. Glucose, blood urea nitrogen (BUN), creatinine, and total bilirubin concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment four

Horse	Density	Water	Sample	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)
1	H	No	1	77	22.2	0.7	0.2
1	H	No	2	128	16.1	0.7	0.8
2	M	Yes	1	75	20.1	0.8	0.3
2	M	Yes	2	119	20.3	1.2	1.1
3	M	Yes	1	88	23.4	1.5	2.4
3	M	Yes	2	103	24.9	1.6	3.8
4	H	No	1	90	19.7	1.1	1.3
4	H	No	2	224	18.3	1.2	5.9
5	H	No	1	92	20.2	1.0	1.0
5	H	No	2	120	23.3	1.6	3.7
6	H	No	1	82	20.3	0.7	0.7
6	H	No	2	103	23.6	1.0	1.9
7	H	No	1	86	20.4	1.0	0.4
7	H	No	2	127	20.9	1.2	1.9
8	L	Yes	1	88	14.0	0.8	1.5
8	L	Yes	2	110	11.6	1.0	2.8
9	M	Yes	1	94	16.6	0.9	0.6
9	M	Yes	2	95	16.9	1.2	2.3
10	L	Yes	1	97	25.1	1.2	0.6
10	L	Yes	2	136	20.1	1.3	1.8
11	L	Yes	1	85	21.9	1.0	0.4
11	L	Yes	2	112	18.7	1.1	1.2
12	M	Yes	1	94	20.1	0.8	0.5
12	M	Yes	2	107	22.6	1.0	1.2
13	L	Yes	1	80	16.2	0.8	0.4
13	L	Yes	2	117	14.5	0.9	1.0
14	L	Yes	1	83	53.9	2.7	0.4
14	L	Yes	2	78	57.3	3.3	0.4
15	M	Yes	1	91	21.5	0.9	0.5
15	M	Yes	2	117	20.1	1.2	3.7

Table A18. Direct bilirubin, alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment four

Horse	Density	Water	Sample	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)	AST (U/l)
1	H	No	1	0.0	104	267	230
1	H	No	2	0.2	128	278	294
2	M	Yes	1	0.1	136	228	252
2	M	Yes	2	0.1	156	189	285
3	M	Yes	1	0.2	122	194	391
3	M	Yes	2	0.2	136	278	422
4	H	No	1	0.3	121	150	178
4	H	No	2	0.2	160	3764	322
5	H	No	1	0.3	117	131	179
5	H	No	2	0.2	134	146	203
6	H	No	1	0.2	122	154	114
6	H	No	2	0.2	127	228	108
7	H	No	1	0.1	180	153	185
7	H	No	2	0.2	211	153	215
8	L	Yes	1	0.2	65	275	896
8	L	Yes	2	0.2	83	163	858
9	M	Yes	1	0.1	173	130	163
9	M	Yes	2	0.2	191	123	184
10	L	Yes	1	0.1	188	317	361
10	L	Yes	2	0.1	193	363	361
11	L	Yes	1	0.1	119	91	197
11	L	Yes	2	0.3	126	100	205
12	M	Yes	1	0.1	194	119	264
12	M	Yes	2	0.2	193	151	269
13	L	Yes	1	0.1	249	535	261
13	L	Yes	2	0.3	207	189	282
14	L	Yes	1	0.1	166	196	126
14	L	Yes	2	0.1	161	197	152
15	M	Yes	1	0.2	206	371	323
15	M	Yes	2	0.1	233	311	366

Table A19. Globulins, albumin/globulin (A/G) ratio, gamma glutamyl transferase (GGT), and cortisol concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment four

Horse	Density	Water	Sample	Globulins (g/dL)	A/G Ratio	GGT (U/l)	Cortisol (ng/mL)
1	H	No	1	5.4	0.43	7	110.4
1	H	No	2	6.6	0.42	9	118.8
2	M	Yes	1	4.0	0.75	7	25.6
2	M	Yes	2	4.4	0.75	11	96.0
3	M	Yes	1	4.8	0.63	6	29.6
3	M	Yes	2	5.3	0.60	6	45.5
4	H	Yes	1	4.0	0.75	14	55.6
4	H	Yes	2	4.7	0.72	18	134.8
5	H	No	1	3.8	0.79	9	43.8
5	H	No	2	4.4	0.75	11	73.0
6	H	No	1	3.7	0.86	9	108.2
6	H	No	2	4.1	0.80	11	104.0
7	H	No	1	3.9	0.72	10	33.6
7	H	No	2	4.5	0.73	12	60.8
8	L	Yes	1	5.6	0.46	8	47.7
8	L	Yes	2	6.2	0.47	10	60.1
9	M	Yes	1	5.6	0.45	7	7.8
9	M	Yes	2	5.9	0.49	10	38.0
10	L	Yes	1	3.8	0.82	9	59.6
10	L	Yes	2	3.8	0.84	10	54.3
11	L	Yes	1	6.0	0.48	9	33.5
11	L	Yes	2	5.9	0.49	10	42.1
12	M	Yes	1	4.9	0.57	61	30.5
12	M	Yes	2	5.0	0.54	60	29.9
13	L	Yes	1	5.2	0.54	23	77.7
13	L	Yes	2	5.3	0.57	15	83.1
14	L	Yes	1	7.1	0.28	10	50.7
14	L	Yes	2	7.5	0.28	10	39.0
15	M	Yes	1	4.3	0.79	12	92.9
15	M	Yes	2	4.5	0.80	11	105.1

Table A20. Electrolyte and aldosterone concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment four

Horse	Density	Water	Sample	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)
1	H	No	1	135	----	101	51.2
1	H	No	2	135	4.2	106	126.5
2	M	Yes	1	138	4.2	102	18.5
2	M	Yes	2	138	4.3	104	99.4
3	M	Yes	1	136	4.0	104	39.3
3	M	Yes	2	138	4.2	105	----
4	H	No	1	141	3.9	107	3.9
4	H	No	2	137	4.3	103	31.2
5	H	No	1	142	3.9	106	59.3
5	H	No	2	140	4.2	105	27.6
6	H	No	1	139	4.0	106	3.1
6	H	No	2	137	3.9	105	9.5
7	H	No	1	140	4.3	107	32.4
7	H	No	2	140	4.3	111	38.9
8	L	Yes	1	134	3.9	101	10.0
8	L	Yes	2	135	3.9	104	76.3
9	M	Yes	1	139	3.9	105	14.5
9	M	Yes	2	138	3.6	106	69.6
10	L	Yes	1	138	4.3	100	35.0
10	L	Yes	2	139	3.8	104	169.6
11	L	Yes	1	139	4.3	105	52.5
11	L	Yes	2	136	4.1	106	2.5
12	M	Yes	1	141	4.1	104	23.2
12	M	Yes	2	136	3.9	103	4.3
13	L	Yes	1	142	----	106	25.6
13	L	Yes	2	132	4.9	96	14.9
14	L	Yes	1	131	4.7	95	93.7
14	L	Yes	2	138	3.5	104	96.6
15	M	Yes	1	138	4.4	103	82.1
15	M	Yes	2	136	4.0	102	88.4

Table A21. Serum protein, albumin, calcium (Ca), and phosphorus (P) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment five

Horse	Density	Water	Sample	Serum Protein (g/dL)	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)
1	H	Yes	1	8.3	3.3	11.4	3.7
1	H	Yes	2	8.9	3.6	11.2	3.9
2	M	No	1	7.0	3.3	11.0	2.1
2	M	No	2	7.6	3.6	10.6	3.2
3	H	Yes	1	7.2	3.0	10.1	3.3
3	H	Yes	2	7.8	3.3	10.2	4.8
4	L	Yes	1	7.6	3.4	11.2	2.7
4	L	Yes	2	8.1	3.6	11.2	3.8
5	H	Yes	1	7.5	3.4	10.4	2.7
5	H	Yes	2	7.4	3.4	10.7	4.6
6	M	No	1	7.0	3.4	11.2	4.9
6	M	No	2	7.7	3.7	9.1	5.3
7	H	Yes	1	7.4	3.2	10.6	4.0
7	H	Yes	2	7.5	3.4	10.2	4.9
8	L	Yes	1	8.8	2.6	10.7	4.9
8	L	Yes	2	8.6	2.5	10.1	4.5
9	H	Yes	1	7.9	2.9	10.3	3.8
9	H	Yes	2	8.7	3.3	9.4	5.3
10	M	No	1	8.4	2.6	10.9	4.8
10	M	No	2	8.7	2.7	9.5	6.6
11	L	Yes	1	7.6	3.1	10.9	4.1
11	L	Yes	2	7.9	3.2	10.7	5.5
12	M	No	1	7.9	2.9	11.0	2.7
12	M	No	2	8.1	3.1	9.6	5.6
13	L	Yes	1	7.2	3.0	10.8	2.8
13	L	Yes	2	7.9	3.2	9.7	3.6
14	M	No	1	7.7	3.1	11.8	4.4
14	M	No	2	8.1	3.3	9.0	4.2
15	L	Yes	1	7.4	3.2	11.6	2.8
15	L	Yes	2	7.8	3.3	9.1	4.0

Table A22. Glucose, blood urea nitrogen (BUN), creatinine, and total bilirubin concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment five

Horse	Density	Water	Sample	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)
1	H	Yes	1	84	20.8	1.5	1.6
1	H	Yes	2	116	20.9	1.6	4.1
2	M	No	1	99	21.3	1.3	0.9
2	M	No	2	134	19.4	1.4	2.5
3	H	Yes	1	99	17.5	0.9	1.8
3	H	Yes	2	123	17.6	1.0	3.5
4	L	Yes	1	87	18.8	1.2	1.2
4	L	Yes	2	96	17.7	1.3	3.0
5	H	Yes	1	117	14.8	1.2	3.3
5	H	Yes	2	139	16.1	1.3	4.4
6	M	No	1	90	21.1	1.1	1.5
6	M	No	2	91	22.2	1.2	3.7
7	H	Yes	1	94	21.3	1.3	1.9
7	H	Yes	2	136	20.1	1.3	3.6
8	L	Yes	1	89	12.3	1.0	0.8
8	L	Yes	2	103	20.1	1.4	1.5
9	H	Yes	1	95	21.6	1.2	2.9
9	H	Yes	2	148	26.5	1.3	5.2
10	M	No	1	98	19.6	1.0	1.5
10	M	No	2	86	22.2	1.1	2.8
11	L	Yes	1	88	18.7	1.2	1.2
11	L	Yes	2	107	19.0	1.4	3.0
12	M	No	1	88	17.3	1.1	1.0
12	M	No	2	112	17.3	1.2	2.2
13	L	Yes	1	81	20.7	1.1	1.1
13	L	Yes	2	92	20.6	1.1	2.2
14	M	No	1	87	20.9	0.9	0.6
14	M	No	2	101	24.7	1.2	1.5
15	L	Yes	1	94	23.9	0.9	0.9
15	L	Yes	2	135	22.9	1.0	2.2

Table A23. Direct bilirubin, alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment five

Horse	Density	Water	Sample	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)	AST (U/l)
1	H	Yes	1	0.1	97	296	358
1	H	Yes	2	0.2	122	314	407
2	M	No	1	0.2	110	1027	351
2	M	No	2	0.2	153	295	374
3	H	Yes	1	0.2	116	258	284
3	H	Yes	2	0.2	135	231	322
4	L	Yes	1	0.3	87	149	279
4	L	Yes	2	0.1	106	161	306
5	H	Yes	1	0.2	139	108	244
5	H	Yes	2	0.1	164	272	253
6	M	No	1	0.1	166	244	274
6	M	No	2	0.2	198	294	307
7	H	Yes	1	0.1	187	186	229
7	H	Yes	2	0.1	218	167	244
8	L	Yes	1	0.2	119	213	200
8	L	Yes	2	0.1	115	173	191
9	H	Yes	1	0.2	149	659	291
9	H	Yes	2	0.2	194	465	357
10	M	No	1	0.1	93	413	261
10	M	No	2	0.2	105	305	264
11	L	Yes	1	0.2	153	342	261
11	L	Yes	2	0.2	175	212	269
12	M	No	1	0.2	127	254	232
12	M	No	2	0.2	139	251	259
13	L	Yes	1	0.2	141	288	250
13	L	Yes	2	0.2	192	264	267
14	M	No	1	0.2	219	225	248
14	M	No	2	0.2	232	226	271
15	L	Yes	1	0.3	129	709	322
15	L	Yes	2	0.2	153	507	360

Table A24. Globulins, albumin/globulin (A/G) ratio, gamma glutamyl transferase (GGT), and cortisol concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment five

Horse	Density	Water	Sample	Globulins (g/dL)	A/G Ratio	GGT (U/l)	Cortisol (ng/mL)
1	H	Yes	1	5	0.66	7	54.4
1	H	Yes	2	5.3	0.68	12	137.1
2	M	No	1	3.7	0.89	12	67.4
2	M	No	2	4.0	0.90	15	92.4
3	H	Yes	1	4.2	0.71	11	60.7
3	H	Yes	2	4.5	0.73	12	99.1
4	L	Yes	1	4.2	0.81	19	53.2
4	L	Yes	2	4.5	0.80	20	74.2
5	H	Yes	1	4.1	0.83	21	72.9
5	H	Yes	2	4.0	0.85	20	68.5
6	M	No	1	3.6	0.94	12	97.7
6	M	No	2	4.0	0.93	13	163.6
7	H	Yes	1	4.2	0.76	12	71.6
7	H	Yes	2	4.1	0.83	14	103.7
8	L	Yes	1	6.2	0.42	13	18.0
8	L	Yes	2	6.1	0.41	13	47.8
9	H	Yes	1	5.0	0.58	11	55.4
9	H	Yes	2	5.4	0.61	12	127.4
10	M	No	1	5.8	0.45	10	47.7
10	M	No	2	6.0	0.45	12	67.4
11	L	Yes	1	4.5	0.69	9	88.1
11	L	Yes	2	4.7	0.68	10	119.8
12	M	No	1	5.0	0.58	8	55.3
12	M	No	2	5.0	0.62	6	117.0
13	L	Yes	1	4.2	0.71	16	62.6
13	L	Yes	2	4.7	0.68	17	128.8
14	M	No	1	4.6	0.67	16	109.9
14	M	No	2	4.8	0.69	16	214.5
15	L	Yes	1	4.2	0.76	13	115.1
15	L	Yes	2	4.5	0.73	14	235.4

Table A25. Electrolyte and aldosterone concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment five

Horse	Density	Water	Sample	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)
1	H	Yes	1	137	----	103	61.3
1	H	Yes	2	142	3.3	106	84.4
2	M	No	1	139	4.2	104	24.3
2	M	No	2	145	3.4	109	----
3	H	Yes	1	136	3.6	103	7.1
3	H	Yes	2	140	4.0	107	29.2
4	L	Yes	1	139	3.6	105	16.6
4	L	Yes	2	140	4.1	107	32.1
5	H	Yes	1	136	3.4	103	30.6
5	H	Yes	2	140	3.0	105	6.2
6	M	No	1	139	4.1	102	12.0
6	M	No	2	143	3.7	106	60.7
7	H	Yes	1	140	3.9	105	----
7	H	Yes	2	145	3.6	109	6.6
8	L	Yes	1	134	4.5	100	15.0
8	L	Yes	2	134	4.0	99	155.6
9	H	Yes	1	140	3.8	105	24.2
9	H	Yes	2	143	3.5	109	104.4
10	M	No	1	136	4.2	101	47.8
10	M	No	2	140	3.6	106	30.1
11	L	Yes	1	137	4.5	103	37.4
11	L	Yes	2	141	3.3	104	14.3
12	M	No	1	136	4.0	104	8.2
12	M	No	2	139	4.7	107	41.8
13	L	Yes	1	136	4.3	103	113.4
13	L	Yes	2	137	3.6	104	24.9
14	M	No	1	137	4.2	103	11.1
14	M	No	2	141	3.7	106	124.3
15	L	Yes	1	136	4.2	102	16.7
15	L	Yes	2	137	4.1	102	25.0

Table A26. Serum protein, albumin, calcium (Ca), and phosphorus (P) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment six

Horse	Density	Water	Sample	Serum Protein (g/dL)	Albumin (g/dL)	Ca (mg/dL)	P (mg/dL)
1	H	Yes	1	8.1	2.4	10.9	3.2
1	H	Yes	2	8.7	2.6	11.0	5.1
2	L	No	1	7.3	3.3	12.2	2.3
2	L	No	2	7.6	3.5	10.6	5.2
3	M	Yes	1	7.1	3.0	12.2	2.5
3	M	Yes	2	7.6	3.3	11.4	4.5
4	M	Yes	1	7.3	2.8	11.4	1.9
4	M	Yes	2	7.4	3.0	11.5	2.3
5	L	No	1	8.4	3.1	12.3	3.9
5	L	No	2	8.0	3.0	10.9	5.3
6	H	Yes	1	7.7	3.2	11.8	3.3
6	H	Yes	2	7.8	3.3	10.9	4.0
7	L	No	1	7.7	3.3	11.3	3.8
7	L	No	2	8.2	3.6	10.4	4.9
8	M	Yes	1	7.5	3.4	11.3	3.1
8	M	Yes	2	7.8	3.5	11.6	3.4
9	H	Yes	1	7.6	2.7	10.8	3.0
9	H	Yes	2	8.7	3.1	11.8	3.6
10	M	Yes	1	7.9	3.0	11.7	3.1
10	M	Yes	2	8.2	3.1	11.4	4.0
11	L	No	1	7.2	3.0	11.7	1.0
11	L	No	2	7.4	3.2	11.4	2.1
12	H	Yes	1	7.1	2.9	10.9	1.9
12	H	Yes	2	7.4	3.1	10.9	3.5
13	L	No	1	7.2	3.3	11.7	1.6
13	L	No	2	7.8	3.5	11.8	3.3
14	M	Yes	1	7.3	3.2	11.6	2.3
14	M	Yes	2	7.6	3.4	11.2	3.5
15	H	Yes	1	8.1	2.9	11.5	2.0
15	H	Yes	2	8.3	3.1	11.2	2.8

Table A27. Glucose, blood urea nitrogen (BUN), creatinine, and total bilirubin concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment six

Horse	Density	Water	Sample	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)
1	H	Yes	1	84	15.1	0.9	1.1
1	H	Yes	2	100	15.2	1.1	2.3
2	L	No	1	90	25.7	1.0	1.1
2	L	No	2	113	23.3	1.2	3.3
3	M	Yes	1	81	23.9	1.1	0.4
3	M	Yes	2	103	17.8	1.2	1.0
4	M	Yes	1	87	29	1.4	0.9
4	M	Yes	2	104	23.9	1.5	1.7
5	L	No	1	75	27.4	0.8	1.3
5	L	No	2	112	32.5	1.1	2.2
6	H	Yes	1	96	17.1	1.0	1.4
6	H	Yes	2	155	16.2	1.0	3.9
7	L	No	1	111	18.7	0.9	1.2
7	L	No	2	185	15.0	1.0	4.2
8	M	Yes	1	102	26.6	1.5	2.7
8	M	Yes	2	121	21.9	1.3	3.7
9	H	Yes	1	94	12.9	0.9	1.2
9	H	Yes	2	123	12.5	1.0	2.1
10	M	Yes	1	103	23.7	1.3	2.0
10	M	Yes	2	113	21.8	1.4	3.5
11	L	No	1	113	17.2	1.0	1.1
11	L	No	2	125	20.1	1.1	1.7
12	H	Yes	1	104	24.1	1.4	1.3
12	H	Yes	2	110	18.7	1.3	2.2
13	L	No	1	107	17.8	0.9	1.3
13	L	No	2	114	15.8	0.9	2.2
14	M	Yes	1	98	25.2	1.3	1.4
14	M	Yes	2	130	20.7	1.3	2.2
15	H	Yes	1	101	17.3	1.2	1.1
15	H	Yes	2	121	16.4	1.3	2.7

Table A28. Direct bilirubin, alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment six

Horse	Density	Water	Sample	Direct Bilirubin (mg/dL)	ALP (U/l)	CK (U/l)	AST (U/l)
1	H	Yes	1	0.2	270	337	330
1	H	Yes	2	0.1	290	441	372
2	L	No	1	0.3	125	170	283
2	L	No	2	0.1	127	256	307
3	M	Yes	1	0.1	116	534	285
3	M	Yes	2	0.2	118	693	312
4	M	Yes	1	0.3	109	146	196
4	M	Yes	2	0.1	116	237	203
5	L	No	1	0.1	152	211	278
5	L	No	2	0.1	132	237	264
6	H	Yes	1	0.2	199	637	279
6	H	Yes	2	0.1	214	288	311
7	L	No	1	0.3	197	360	449
7	L	No	2	0.1	213	328	491
8	M	Yes	1	0.2	122	223	293
8	M	Yes	2	0.1	128	218	302
9	H	Yes	1	0.1	242	248	211
9	H	Yes	2	0.1	276	352	245
10	M	Yes	1	0.2	175	193	228
10	M	Yes	2	0.1	180	268	246
11	L	No	1	0.2	219	367	392
11	L	No	2	0.1	233	281	386
12	H	Yes	1	0.2	96	708	301
12	H	Yes	2	0.1	99	563	340
13	L	No	1	0.2	172	441	343
13	L	No	2	0.1	182	373	357
14	M	Yes	1	0.1	165	376	431
14	M	Yes	2	0.1	169	284	447
15	H	Yes	1	0.2	307	182	223
15	H	Yes	2	0.1	315	188	231

Table A29. Globulins, albumin/globulin (A/G) ratio, gamma glutamyl transferase (GGT), and cortisol concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment six

Horse	Density	Water	Sample	Globulins (g/dL)	A/G Ratio	GGT (U/l)	Cortisol (ng/mL)
1	H	Yes	1	5.7	0.42	43	23.7
1	H	Yes	2	6.1	0.43	44	83.0
2	L	No	1	4.0	0.82	9	125.6
2	L	No	2	4.1	0.85	6	165.5
3	M	Yes	1	4.1	0.73	11	135.4
3	M	Yes	2	4.3	0.77	9	141.3
4	M	Yes	1	4.5	0.62	27	64.5
4	M	Yes	2	4.4	0.68	27	34.0
5	L	No	1	5.3	0.58	39	76.1
5	L	No	2	5.0	0.60	33	98.4
6	H	Yes	1	4.5	0.71	18	108.8
6	H	Yes	2	4.5	0.73	15	24.9
7	L	No	1	4.4	0.75	31	63.5
7	L	No	2	4.6	0.78	31	34.3
8	M	Yes	1	4.1	0.83	41	110.5
8	M	Yes	2	4.3	0.81	38	52.7
9	H	Yes	1	4.9	0.55	14	36.0
9	H	Yes	2	5.6	0.55	13	76.5
10	M	Yes	1	4.9	0.61	13	56.9
10	M	Yes	2	5.1	0.61	12	74.9
11	L	No	1	4.2	0.71	11	59.9
11	L	No	2	4.2	0.76	9	71.2
12	H	Yes	1	4.2	0.69	13	54.2
12	H	Yes	2	4.3	0.72	8	39.5
13	L	No	1	3.9	0.85	19	77.9
13	L	No	2	4.3	0.81	18	58.9
14	M	Yes	1	4.1	0.78	15	169.6
14	M	Yes	2	4.2	0.81	11	253.2
15	H	Yes	1	5.2	0.56	11	42.8
15	H	Yes	2	5.2	0.60	11	27.3

Table A30. Electrolyte and aldosterone concentrations for each horse in the different density (high = H, medium = M, low = L) and water (water access = Yes, no water access = No) treatments pre- (sample 1) and post- (sample 2) transport for shipment six

Horse	Density	Water	Sample	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Aldosterone (ng/dL)
1	H	Yes	1	134	4.2	104	56.6
1	H	Yes	2	135	4.8	107	42.0
2	L	No	1	135	4.5	104	42.8
2	L	No	2	137	4.9	107	49.0
3	M	Yes	1	137	4.4	104	39.9
3	M	Yes	2	137	4.5	106	----
4	M	Yes	1	136	4.2	104	28.7
4	M	Yes	2	135	4.7	106	36.9
5	L	No	1	135	4.8	104	66.3
5	L	No	2	137	5.1	107	5.2
6	H	Yes	1	137	3.9	105	57.7
6	H	Yes	2	136	4.5	106	15.4
7	L	No	1	137	4.1	105	5.9
7	L	No	2	137	4.1	106	13.5
8	M	Yes	1	137	3.5	102	14.8
8	M	Yes	2	137	4.5	103	43.5
9	H	Yes	1	135	4.4	106	56.9
9	H	Yes	2	136	5.2	105	65.5
10	M	Yes	1	135	4.3	104	76.8
10	M	Yes	2	133	5.5	105	24.3
11	L	No	1	137	4.4	105	4.0
11	L	No	2	137	4.8	107	10.1
12	H	Yes	1	136	4.1	102	28.5
12	H	Yes	2	132	5.6	101	29.6
13	L	No	1	137	4.2	106	6.5
13	L	No	2	138	4.6	107	58.8
14	M	Yes	1	138	4.2	104	34.3
14	M	Yes	2	137	5.6	106	59.5
15	H	Yes	1	136	4.5	105	81.7
15	H	Yes	2	135	4.3	106	----

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